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(54) **SYSTEMS AND METHODS USING SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR DETECTING TETRAHYDROCANNABINOL**

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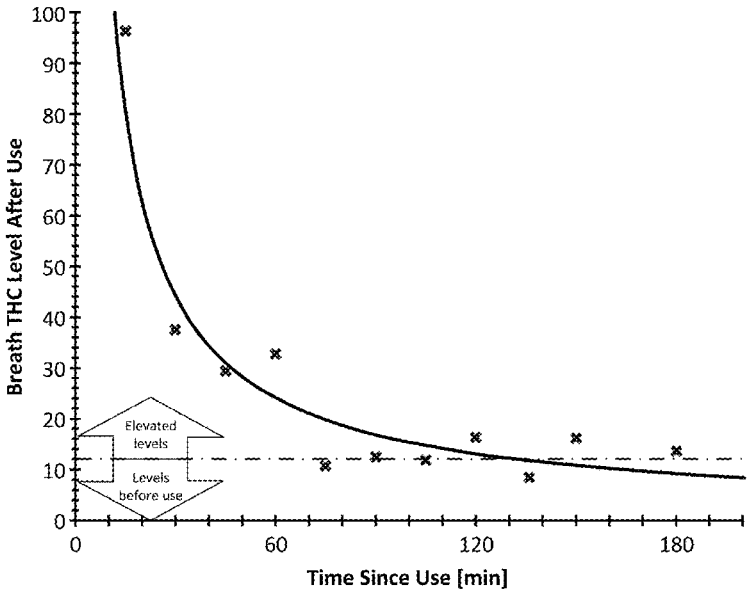
(57) **ABSTRACT**

The present disclosure relates to using Surface-Enhanced Raman Spectroscopy (SERS) for detecting analytes in samples. Uses can include, for example, detection of tetrahydrocannabinol (THC) using SERS, as well as apparatuses and systems to implement such detection methods.

21 Claims, 12 Drawing Sheets



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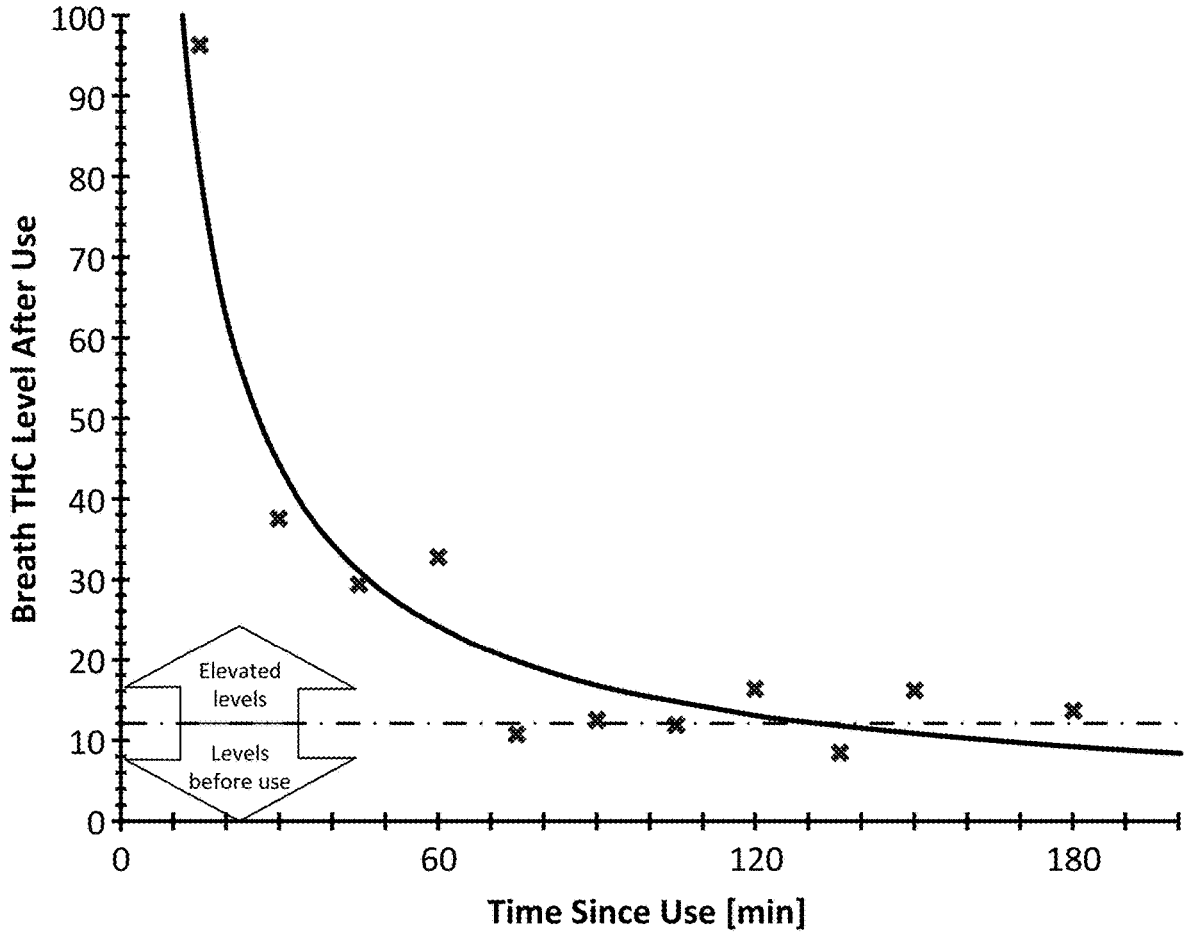
Breath THC Level After Use
100
90
80
70
60
50
40
30
20
10
0
Elevated levels
Levels before use
0
60
120
180
Time Since Use [min]

FIG. 1

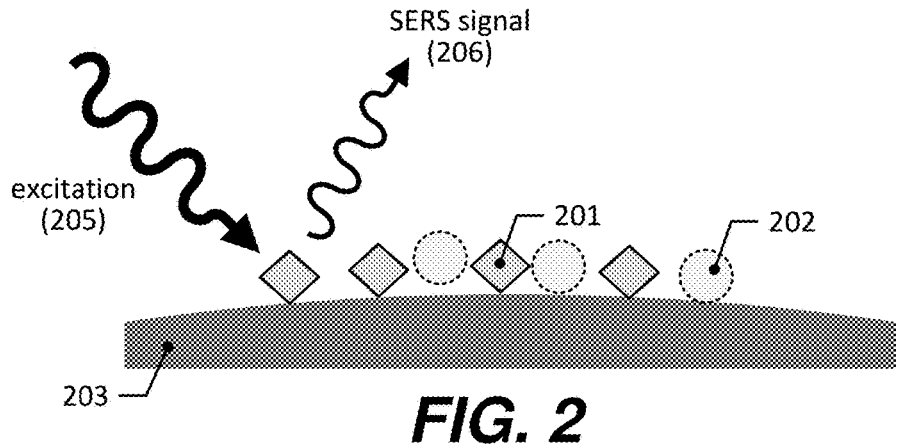
SERS signal (206)
excitation (205)
201
202
203

FIG. 2

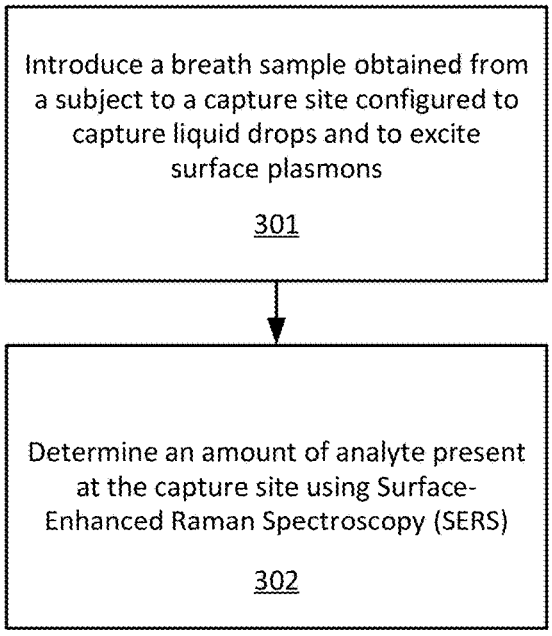
Introduce a breath sample obtained from a subject to a capture site configured to capture liquid drops and to excite surface plasmons
301
Determine an amount of analyte present at the capture site using Surface-Enhanced Raman Spectroscopy (SERS)
302

FIG. 3A

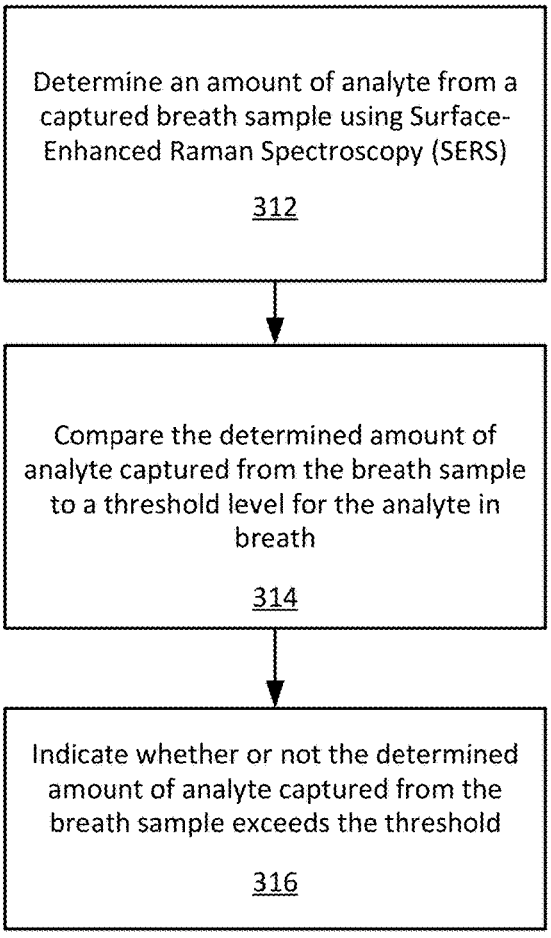
Determine an amount of analyte from a captured breath sample using Surface-Enhanced Raman Spectroscopy (SERS)
312
Compare the determined amount of analyte captured from the breath sample to a threshold level for the analyte in breath
314
Indicate whether or not the determined amount of analyte captured from the breath sample exceeds the threshold
316

FIG. 3B

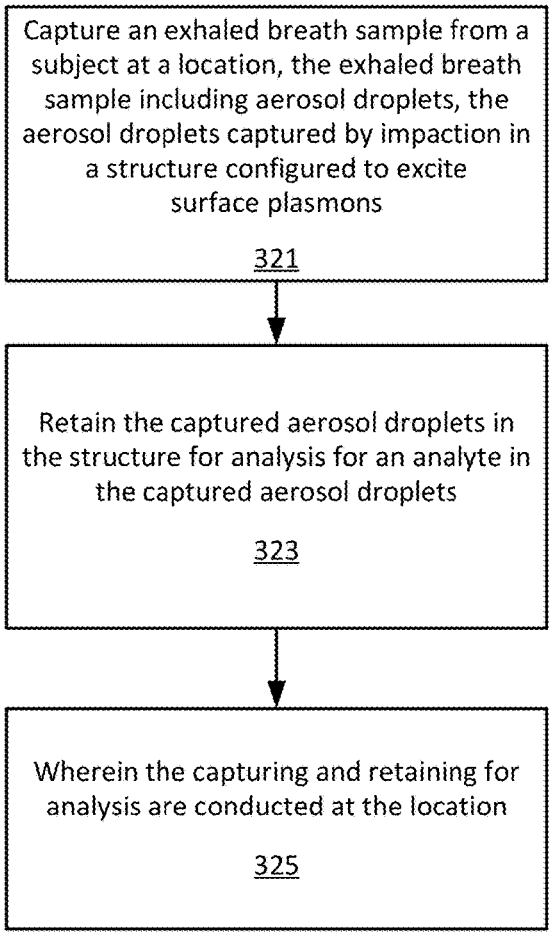
Capture an exhaled breath sample from a subject at a location, the exhaled breath sample including aerosol droplets, the aerosol droplets captured by impaction in a structure configured to excite surface plasmons
321
Retain the captured aerosol droplets in the structure for analysis for an analyte in the captured aerosol droplets
323
Wherein the capturing and retaining for analysis are conducted at the location
325

FIG. 3C

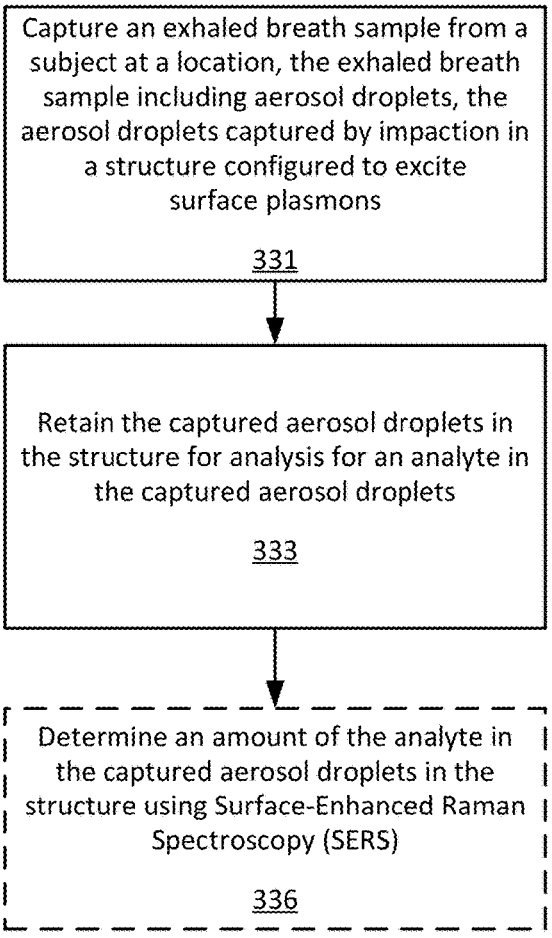
Capture an exhaled breath sample from a subject at a location, the exhaled breath sample including aerosol droplets, the aerosol droplets captured by impaction in a structure configured to excite surface plasmons
331
Retain the captured aerosol droplets in the structure for analysis for an analyte in the captured aerosol droplets
333
Determine an amount of the analyte in the captured aerosol droplets in the structure using Surface-Enhanced Raman Spectroscopy (SERS)
336

FIG. 3D

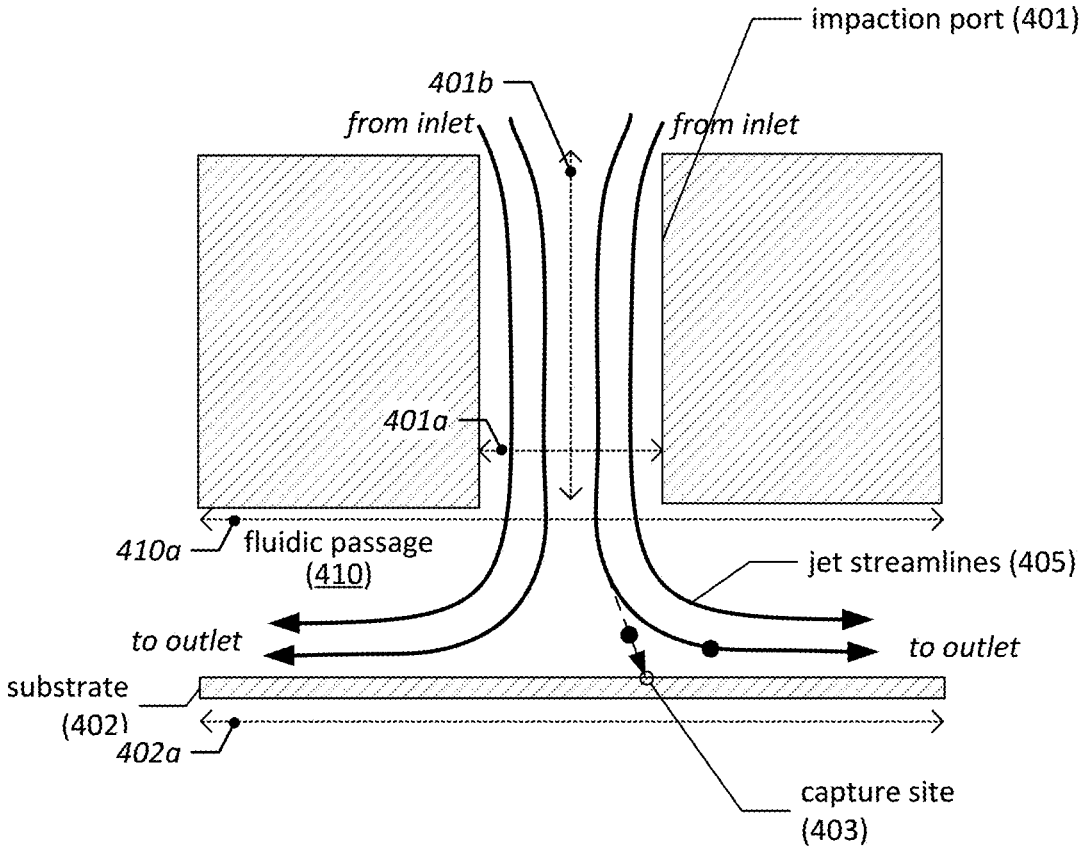
impaction port (401)
401b
from inlet
from inlet
401a
410a
fluidic passage
(410)
jet streamlines (405)
to outlet
to outlet
substrate
(402)
402a
capture site
(403)

FIG. 4A

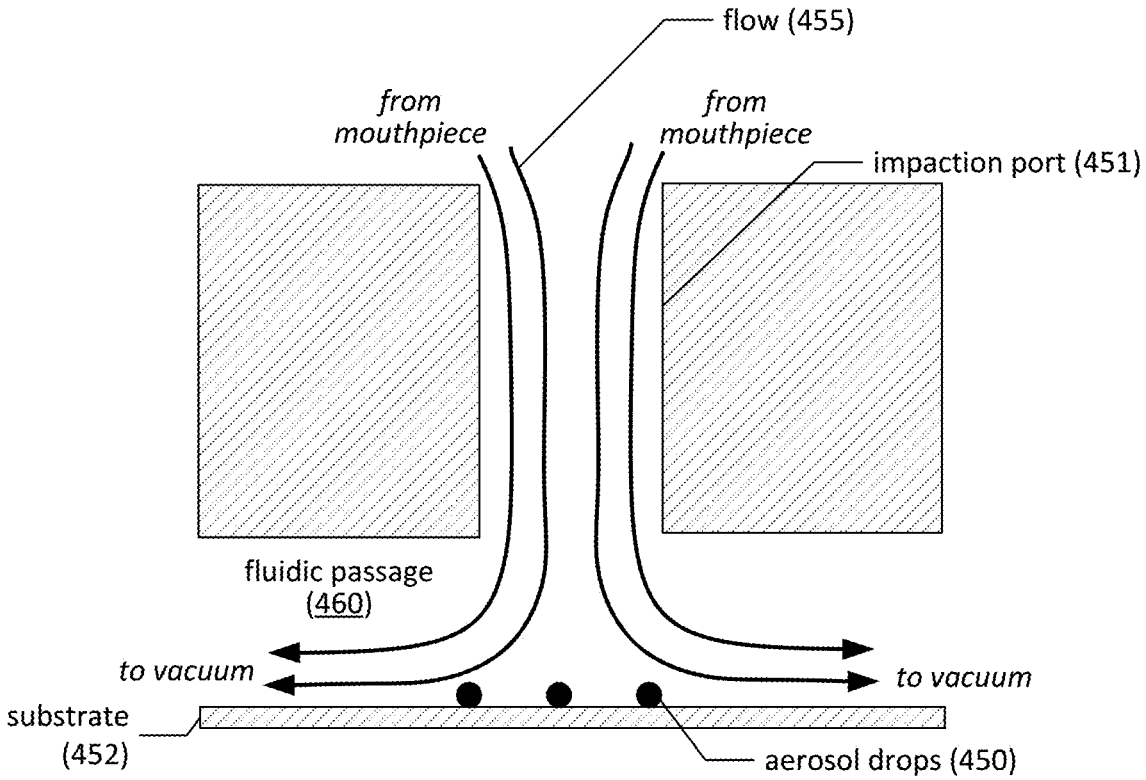
flow (455)
from mouthpiece
from mouthpiece
impaction port (451)
fluidic passage (460)
to vacuum
to vacuum
substrate (452)
aerosol drops (450)

FIG. 4B

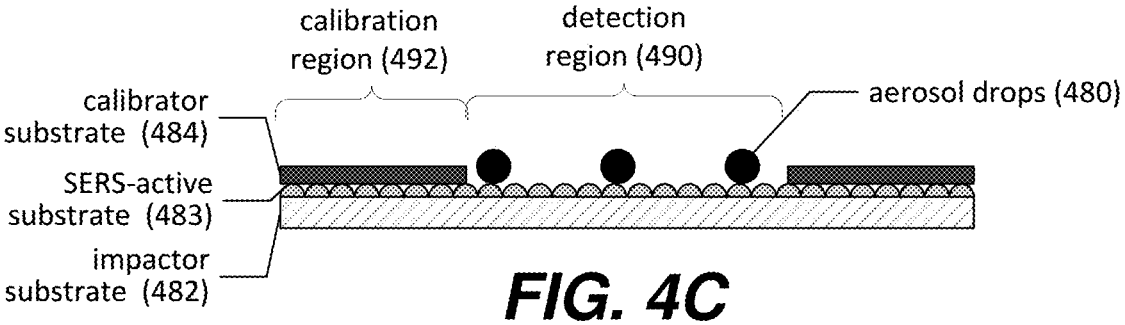
calibration region (492)
detection region (490)
aerosol drops (480)
calibrator substrate (484)
SERS-active substrate (483)
impactor substrate (482)

FIG. 4C

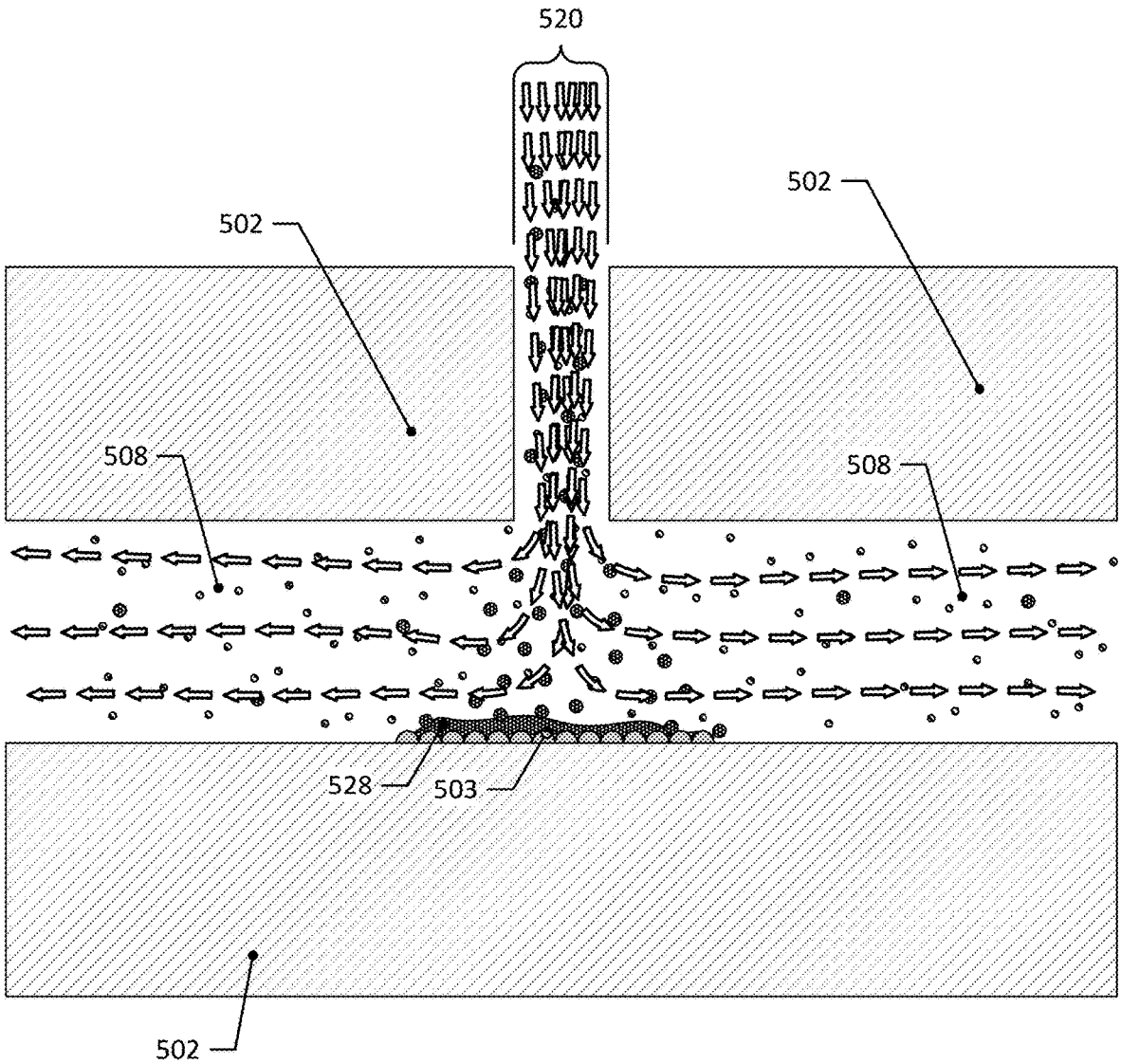

FIG. 5

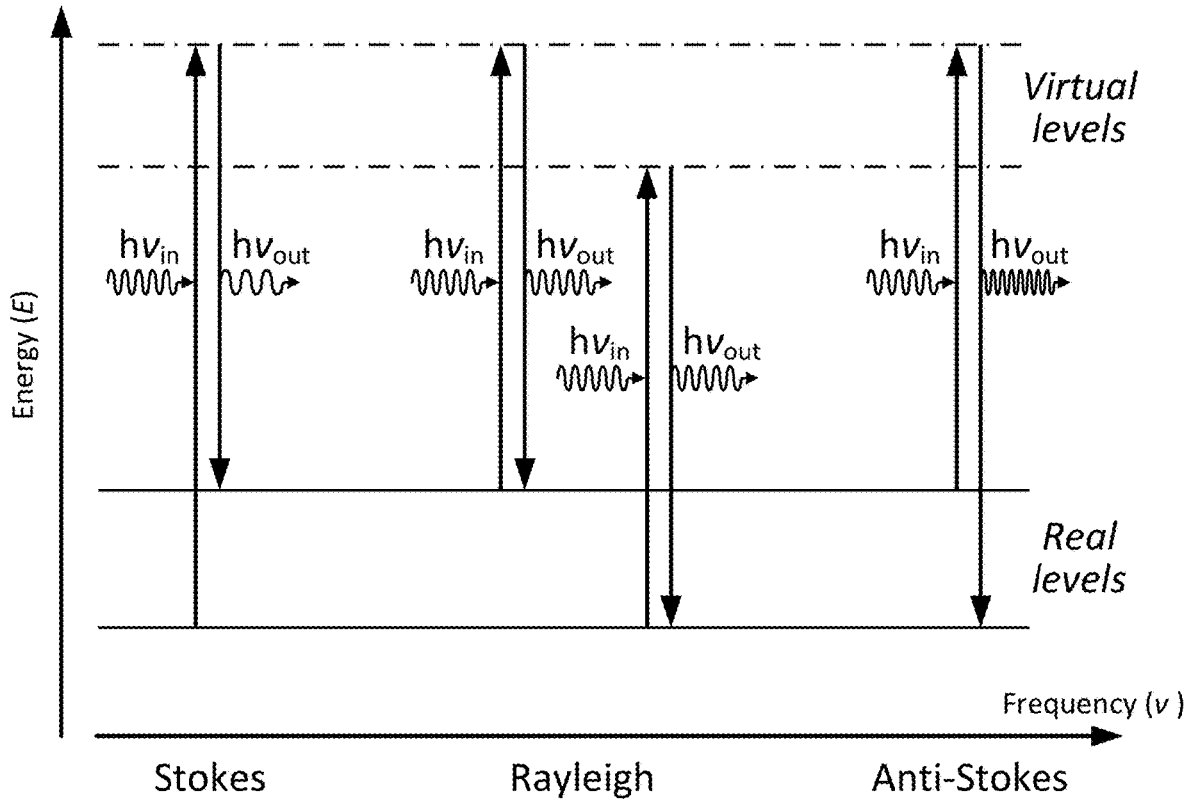
Virtual levels
Real levels
Energy (E)
Frequency (v)
hvin
hvout
hvin
hvout
hvin
hvout
hvin
hvout
Stokes
Rayleigh
Anti-Stokes

FIG. 6

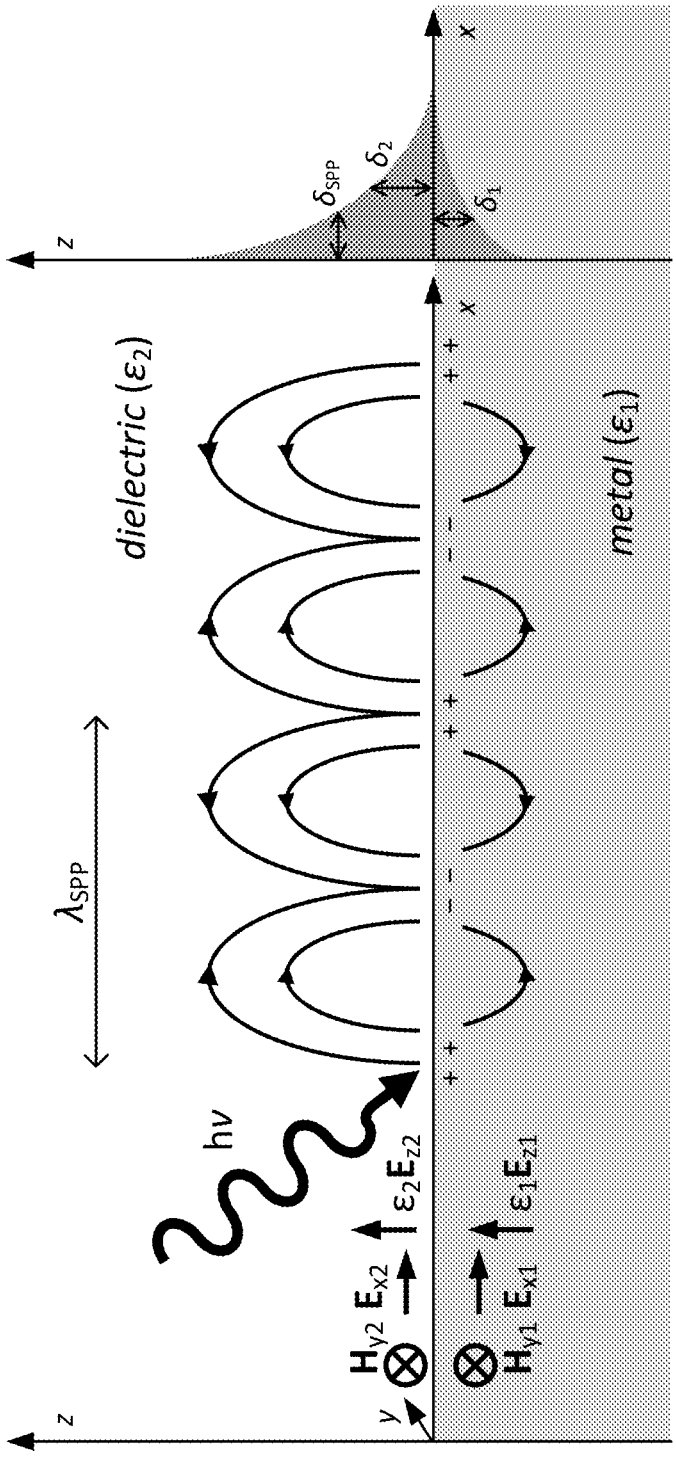
z
x
δ_{SPP}
δ_2
δ_1
dielectric (ε_2)
metal (ε_1)
λ_{SPP}
hν
H_{y2} E_{x2} $\varepsilon_2 E_{z2}$
H_{y1} E_{x1} $\varepsilon_1 E_{z1}$
y

FIG. 7

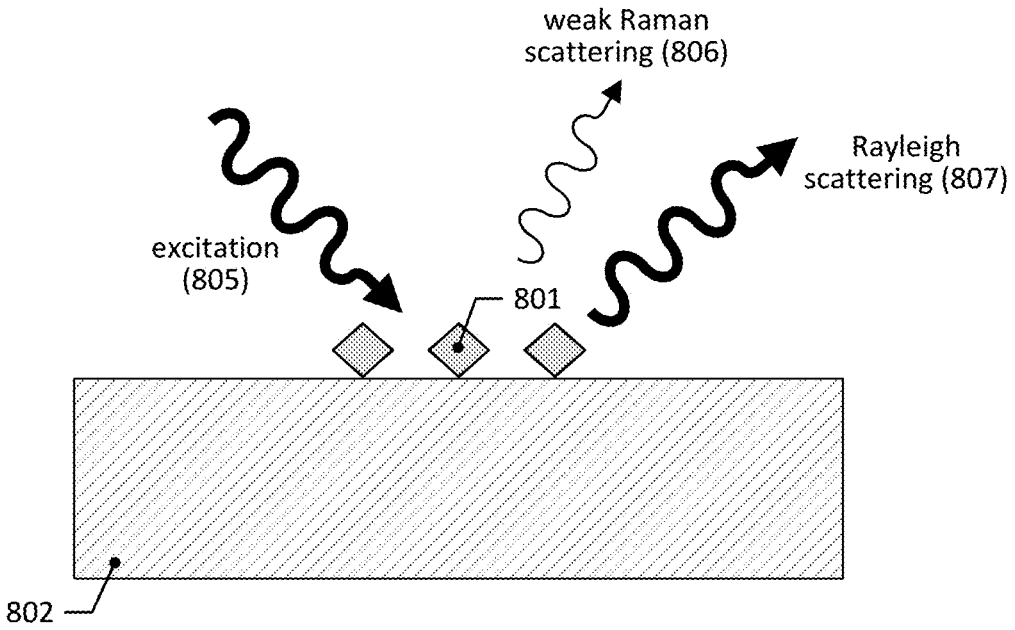
weak Raman
scattering (806)
Rayleigh
scattering (807)
excitation
(805)
801
802

FIG. 8A

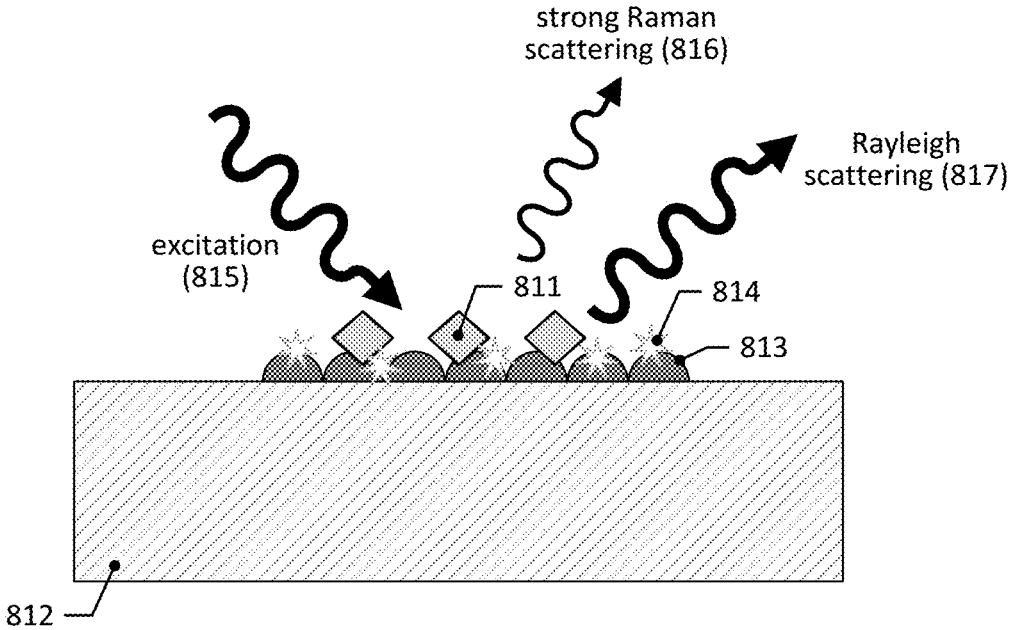
strong Raman
scattering (816)
Rayleigh
scattering (817)
excitation
(815)
811
814
813
812

FIG. 8B

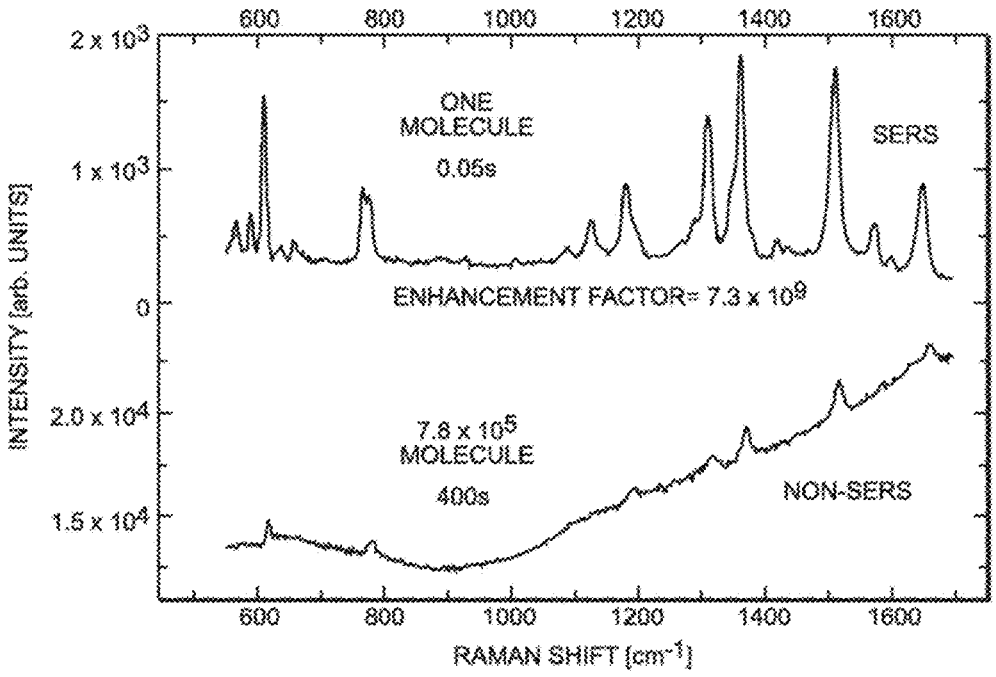
600
800
1000
1200
1400
1600
2 x 10^3
1 x 10^3
0
2.0 x 10^4
1.5 x 10^4
INTENSITY [arb. UNITS]
ONE MOLECULE
0.05s
SERS
ENHANCEMENT FACTOR= 7.3 x 10^9
7.8 x 10^5 MOLECULE
400s
NON-SERS
RAMAN SHIFT [cm^-1]

FIG. 9

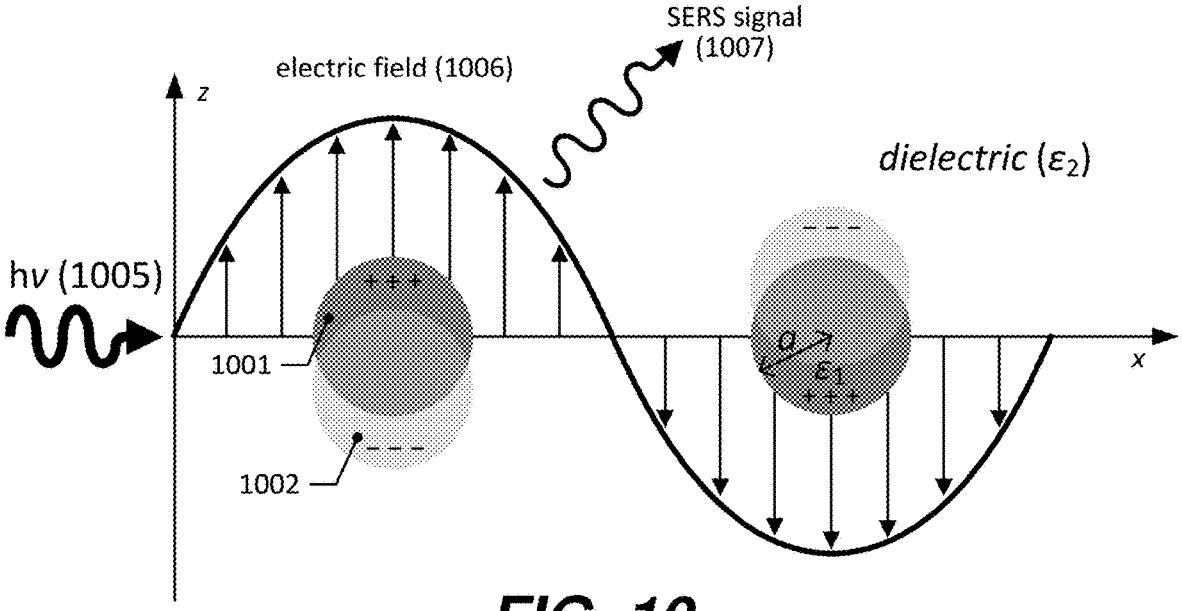
SERS signal (1007)
electric field (1006)
z
dielectric (ε2)
hν (1005)
1001
1002
Q
ε1
x

FIG. 10

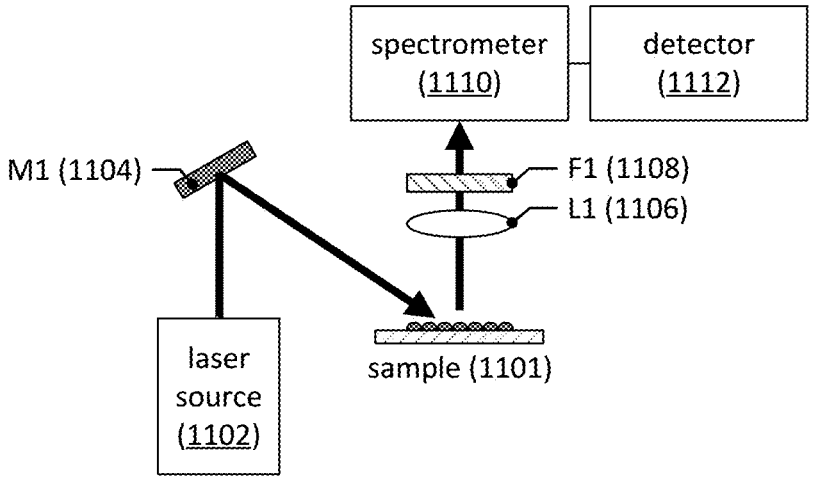

FIG. 11A

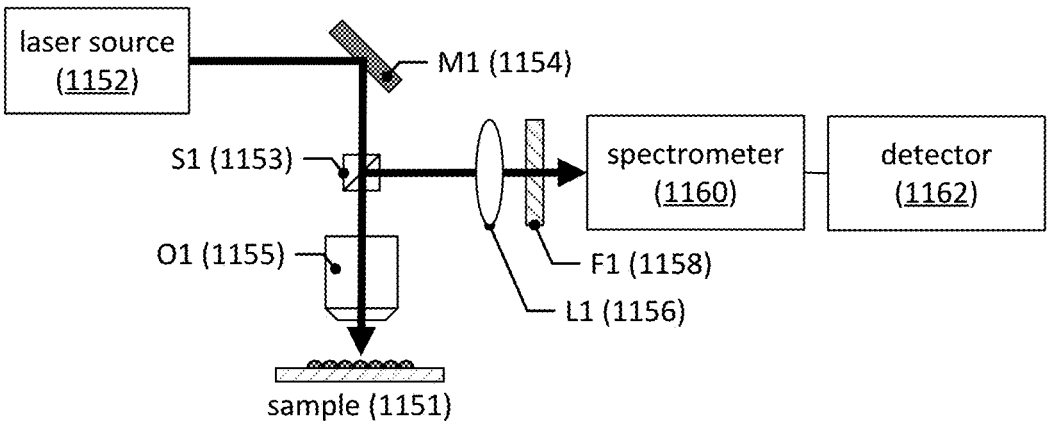

FIG. 11B

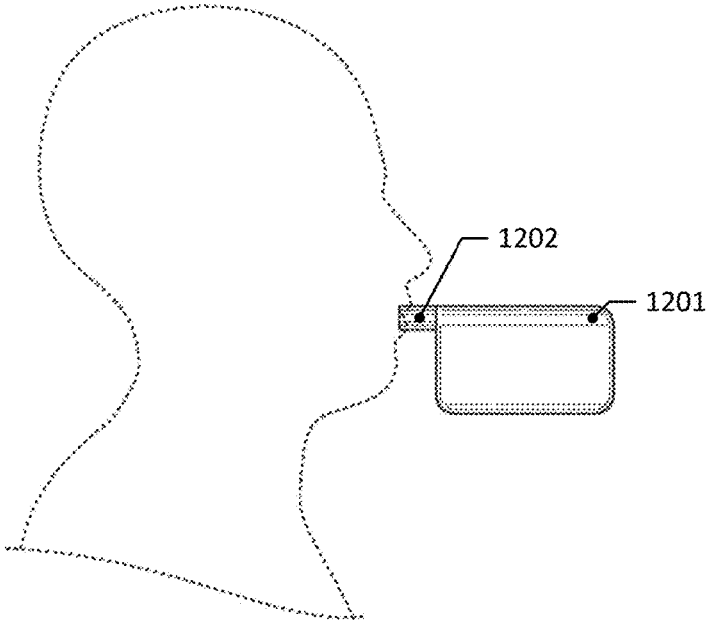

FIG. 12A

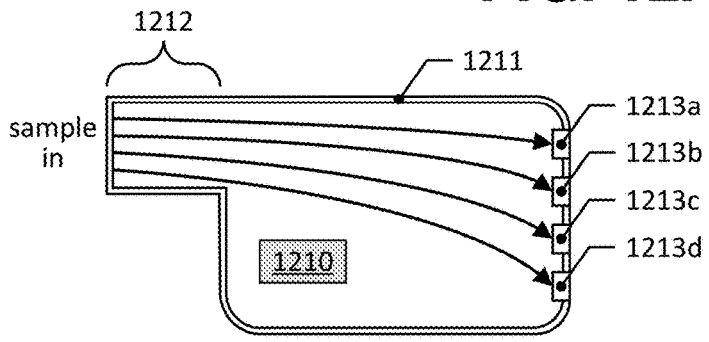

FIG. 12B

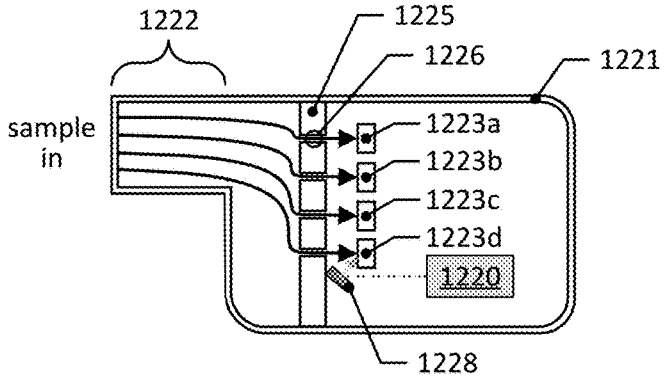

FIG. 12C

SYSTEMS AND METHODS USING SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR DETECTING TETRAHYDROCANNABINOL

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application No. 63/024,423, filed May 13, 2020, which is incorporated herein by reference in its entirety.

TECHNICAL FIELD

The present technology pertains to using Surface-Enhanced Raman Spectroscopy (SERS) for detecting analytes in samples. In particular, but not by way of limitation, the present technology provides systems and methods using SERS for detecting cannabinoids including tetrahydrocannabinol (THC) in breath samples.

BACKGROUND

With legalization of marijuana expanding and the risk of marijuana-associated impaired driving increasing, there is a need for additional methods and devices for determining levels of cannabinoid compound, such as tetrahydrocannabinol (THC) in a subject's sample.

The background description provided herein is for the purposes of generally presenting the context of the disclosure. Work of the presently named inventors, to the extent it is described in this background section, as well as aspects of the description that may not otherwise qualify as prior art at the time of filing, are neither expressly nor impliedly admitted as prior art against the present disclosure.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described in the Detailed Description section below. This summary is not intended to identify key features or essential features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

The present disclosure relates to using Surface-Enhanced Raman Spectroscopy (SERS) for detecting analytes in samples. Uses can include detection of a cannabinoid using SERS, as well as apparatuses and systems to implement such detection methods. In particular embodiments, detecting can include determining an amount of tetrahydrocannabinol (THC) in a breath sample from a subject.

Accordingly, in a first aspect, the present disclosure encompasses a handheld breath sample apparatus for detection of an analyte in a breath sample SERS.

In some embodiments, the apparatus includes: a housing, in which the housing includes a cartridge interface configured to mechanically interface with a cartridge that has a SERS-active substrate. In particular embodiments, the SERS-active substrate including one or more capture sites for a breath sample.

In some embodiments, the analyte present in the breath sample is absorbed or adsorbed at or in the capture sites. In particular embodiments, the capture site is or includes a metal nanostructure, which in turn is disposed on a surface configured to capture an aerosol drop from a breath sample by impaction.

In some embodiments, a surface of the SERS-active substrate is configured to cause excitement of surface plasmons (e.g., localized surface plasmons) upon exposure to a laser light, thereby enhancing Raman signals and allowing for trace detection of the analyte. In particular embodiments, the analyte is THC or an analog thereof.

In other embodiments, the enhancing of the Raman signals provides a 10^3 to 10^{10}-fold signal increase, as compared to traditional "bulk" Raman scattering, by a strong electromagnetic wave coupling of the Raman signals.

In some embodiments, the trace detection of the analyte is single molecule detection of the analyte in the breath sample.

In other embodiments, the SERS-active substrate is configured to facilitate droplet capture through inertial impaction. In one instance, the apparatus further includes an impaction port disposed within the housing, wherein the impaction port has a longitudinal axis that is perpendicular to a major plane of the SERS-active substrate, and wherein the impaction port is configured to be in fluidic communication with the one or more capture sites disposed on a surface of the SERS-active substrate. In yet other embodiments, the SERS-active substrate further includes a detection region (e.g., configured to capture one or more aerosol drops) and a calibration region (e.g., configured to provide a layer of a surfactant present in the aerosol drops).

In some embodiments, the apparatus further include an interface or a laser source configured to optically access the one or more capture sites. In particular embodiments, the interface can be configured to optically couple to a detection device (e.g., in which the interface allows for a spectrometer or detector configured to optically access the capture sites(s) and/or SERS-active substrate(s)).

In a second aspect, the present disclosure encompasses a method using SERS for detection of an analyte in a breath sample. The method can include: determining an amount of analyte captured from a breath sample using SERS using a SERS-active substrate, the SERS-active substrate including one or more capture sites for a breath sample; comparing the determined amount of analyte captured from the breath sample to a threshold level for the analyte in breath; and indicating whether the determined amount of the analyte captured from the breath sample exceeds the threshold.

In some embodiments, the determining an amount of the analyte captured from a breath sample using SERS includes receiving enhanced Raman signals that is 10^3 to 10^{10} fold signal increase, as compared to traditional "bulk" Raman scattering, by a strong electromagnetic wave coupling of the enhanced Raman signals.

In particular embodiments, the analyte is THC or an analog thereof. In some embodiments, the threshold is correlated with a baseline maximum level of THC in breath associated with consumption of THC outside a window of THC-associated impairment. In particular embodiments, the threshold is correlated with an average amount of THC in breath between 2 and 3 hours after inhalation.

In some embodiments, the determining the amount of the analyte captured from the breath sample using SERS allows for single molecule sensitivity.

In further embodiments, the method further includes: wirelessly transmitting data corresponding to the determining an amount of the analyte captured from the breath sample using SERS, the comparing the determined amount of the analyte from the breath sample to the threshold level for the analyte in breath, and the indicating whether or not the determined amount of the analyte captured from the breath sample exceeds the threshold, to a remote location.

In some embodiments, the analyte captured from the breath sample using SERS is captured with a hand-held device.

In a third aspect, the present disclosure encompasses a system using SERS for detection of an analyte in a breath sample. In some embodiments, the system includes: an excitation laser configured to excite a SERS-active substrate, the SERS-active substrate including one or more capture sites for the breath sample; a high sensitivity spectrometer configured to collect one or more Raman signals from the one or more capture sites; and a fiber optic Raman probe electrically connected to the high sensitivity spectrometer.

In some embodiments, the one or more Raman signals are directly proportional to an amount of the analyte captured in the breath sample.

In some embodiments, the excitation laser has an irradiation wavelength from about 500-650 nm (e.g., from 500-600 nm, 550-650 nm, or about 600 nm). In other embodiments, the irradiation wavelength is from about 400-550 nm (e.g., from 400-500 nm, 450-500 nm, or about 488 nm).

In further embodiments, the system includes: a cartridge, wherein the cartridge including the SERS-active substrate including the one or more capture sites for the breath sample.

In any embodiment herein, the analyte is tetrahydrocannabinol (THC) or an analog thereof. Non-limiting analogs can include cannabinol (CBN), cannabidiol (CBD), carboxy THC or 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-hydroxy-Δ9-tetrahydrocannabinol (11-hydroxy THC), 9-carboxy THC or Δ9-tetrahydrocannabinolic acid (THC-9-COOH), tetrahydrocannabinolic acid (THCA, THC-2-COOH), as well as isomers thereof.

In any embodiment herein, the SERS-active substrate includes one or more metal nanostructures. Non-limiting nanostructures can include metal nanoparticles, such as gold nanoparticles, silver nanoparticles, and/or copper nanoparticles.

Details of one or more implementations of the subject matter described in this specification are set forth in the accompanying drawings and the description below. Other features, aspects, and advantages will become apparent from the description, the drawings, and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, where like reference numerals refer to identical or functionally similar elements throughout the separate views, together with the detailed description below, are incorporated in and form part of the specification, and serve to further illustrate embodiments of concepts that include the claimed disclosure, and to explain various principles and advantages of those embodiments.

The methods and systems disclosed herein have been represented where appropriate by conventional symbols in the drawings, showing only those specific details that are pertinent to understanding the embodiments of the present disclosure so as not to obscure the disclosure with details that will be readily apparent to those of ordinary skill in the art having the benefit of the description herein.

FIG. **1** depicts a plot showing breath THC level vs. time since use.

FIG. **2** illustrates a non-limiting embodiment for detecting the presence of an analyte **201** by detecting a Surface-Enhanced Raman Spectroscopy (SERS) signal **206**, according to various embodiments of the present technology.

FIG. **3**A-**3**D depicts (A) a non-limiting process flow chart for method in accordance with the present disclosure, (B) another non-limiting process flow chart, (C) yet another non-limiting process flow chart, and (D) a further non-limiting process flow chart.

FIG. **4**A-**4**C shows a cross-sectional diagrams of an inertial impaction structure, that can serve as a non-limiting capture site of the breath collection module (BCM). Provided are (A) a diagram of a non-limiting inertial impaction structure, (B) another diagram of a non-limiting inertial impaction structure; and (C) yet another diagram of a non-limiting inertial impaction structure showing an example of a SERS-active substrate.

FIG. **5** depicts a cross-section view of a portion of a droplet trap.

FIG. **6** illustrates an example of an energy diagram showing Rayleigh scattering and Raman scattering (Stokes and anti-Stokes).

FIG. **7** illustrates an example of a surface plasmon at an interface between a metal (having permittivity ε_1) and a dielectric (having permittivity ε_2), which can be employed for Surface Plasmon Resonance (SPR) according to various embodiments of the present technology.

FIG. **8**A-**8**B shows the extent of Rayleigh and Raman scattering in the presence of an analyte at (A) a first interface and (B) at a second interface for use in Surface-Enhanced Raman Spectroscopy (SERS), according to various embodiments of the present technology.

FIG. **9** illustrates enhanced sensitivity of Surface-Enhanced Raman Spectroscopy (SERS) compared with "bulk" Raman scattering, according to various embodiments of the present technology.

FIG. **10** illustrates a spherical model for Surface-Enhanced Raman Spectroscopy (SERS), according to various embodiments of the present technology.

FIG. **11**A-**11**B illustrates exemplary systems and instrumentation for Surface-Enhanced Raman Spectroscopy (SERS) including (A) a first experimental setup and (B) a second experimental setup, according to various embodiments of the present technology.

FIG. **12**A-**12**C illustrates (A) a view of an exemplary breath capturing device in use by a subject and cross-sectional views of (B) a non-limiting device and (C) another non-limiting device for use with Surface-Enhanced Raman Spectroscopy (SERS) for detecting trace levels of molecules including cannabinoids such as tetrahydrocannabinol (THC) in breath samples, according to various embodiments of the present technology. FIG. **12**A-**12**C are not necessarily drawn to scale.

DETAILED DESCRIPTION

In various embodiments of the present technology, Surface Enhanced Raman Spectroscopy (SERS) is an extension of Raman spectroscopy in which metal structures (e.g., nanoparticles, such as gold or silver nanoparticles) amplify Raman signals. This technique works via an electromagnetic effect where molecules come into proximity with the metal structures. When incident laser light strikes the metal structures or a surface thereof (e.g., a metal nanoparticulate surface), surface plasmons (or localized surface plasmons) are excited, greatly enhancing Raman signals. The enhancement is significant, making SERS capable of trace level detection of molecules or analytes.

SERS-based tetrahydrocannabinol (THC) detection may be performed with urine, blood, or saliva. Breath based detection is typically more difficult, but the capture mecha-

nism of the present technology allows a higher surface density of THC molecules allowing using SERS for detecting THC in breath samples with single molecule detection sensitivity. Furthermore, SERS can allow for label-free detection of analytes, as well as detection of captured, dried samples in some instances. Accordingly, described herein are apparatuses, systems, and methods for capturing breath samples on substrates that facilitate SERS-based detection of analytes.

In particular embodiments, the present disclosure relates to detection in which the analyte is THC or an analog thereof. FIG. **1** depicts a plot showing breath THC level in picograms (pg) per breath (5 L) vs. time in minutes (min) since use in chronic or frequent THC smokers. From the plot it can be seen that THC level in breath drops substantially in the first hour, and after 2 hours it drops below the maximum baseline threshold for chronic users. Testing has determined a maximum baseline THC level in breath for chronic users to be in the picogram per liter of breath range. Based on data obtained through testing, it appears that the threshold may represent a baseline mean level of residual THC in breath associated with consumption of THC across a broad demographic, regardless of use frequency, outside a window from inhalation to between 2 and 3 hours after inhalation, which has been associated with THC impairment. The threshold referenced in the comparison of the disclosed method may be less than 10 picogram/liter (pg/L) of breath, or from 2 to 5 pg/L of breath, or from 2 to 3 pg/L of breath, for example, about 2.4 pg/L of breath. The maximum baseline 12 pg/5 L (2.4 pg/L) breath is superimposed on the plot. The threshold may vary depending upon the capture efficiency of the device or system with which the method is conducted, and can be tuned in practice.

FIG. **2** illustrates a non-limiting embodiment for SERS-based detection of a target analyte **201** (e.g., THC or an analog thereof). In some instances, the target analyte **201** may be associated with other molecules **202**, such as surfactants (e.g., phospholipids) that can be present in aerosolized droplets of exhaled breath samples. SERS-based detection can be employed in the presence of such other molecules **202** without necessary interfering with the detection of the target analyte **201**.

Non-limiting analytes include THC, as well as other markers, such as cannabinol (CBN), cannabidiol (CBD), carboxy THC or 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-hydroxy THC), 9-carboxy THC or Δ^9-tetrahydrocannabinolic acid (THC-9-COOH), tetrahydrocannabinolic acid (THCA, THC-2-COOH), and similar compounds, as well as isomers thereof.

Turning again to FIG. **2**, SERS-based detection includes the use of a SERS-active substrate **203**. Herein, the SERS-active substrate is configured to provide a capture site that can interact with the target analyte, as well as to provide a surface that can propagate or cause excitement of surface plasmons. Detailed description of the SERS-based detection and SERS-active substrates are more fully provided herein.

Surface plasmons are generated by providing an excitation radiation **205** to the SERS-active substrate **203**, thereby generating a SERS signal **206** that can indicate whether or not the target analyte **201** present. The SERS signal can be analyzed, compared, deconvoluted, processed, or otherwise evaluated to determine the presence of the target analyte. In one instance, the measured SERS signal (e.g., a Raman spectrum) is compared to a signature SERS signal that is associated with the target analyte. Such signatures can include the presence of one or more peaks at certain wavelengths or the presence of certain combinations of peaks at certain intensities and/or wavelengths. In particular embodiments, the SERS signal can provide single molecule detection of the target analyte.

Referring to FIG. **3**A, a general flow chart for a method in accordance with the present disclosure is depicted. According to various embodiments, the method for detection of an analyte can include introducing a breath sample obtained from a subject to a capture site **301**; and determining amount of a target analyte present at the capture site using SERS **302**. To facilitate use with a breath sample, the capture site can be configured to capture liquid drops (e.g., using a droplet trap, as described herein). To facilitate use of SERS, the capture site can be configured to excite surface plasmons, such as localized surface plasmons. In particular embodiments, the capture site can include one or more metal structures (e.g., metal nanoparticles or other metal nanostructures).

Such methods can also include comparing an amount of analyte to determine whether or not the determined amount exceeds a particular threshold. As discussed herein with reference to FIG. **1**, such a threshold may be useful when detecting THC and assessing whether a determined amount of THC in the breath sample is associated with THC impairment. Thus, FIG. **3**B shows another general flow chart for a method in accordance with the present disclosure is depicted.

According to various embodiments, the method for detection of an analyte can include determining an amount of a target analyte from a captured breath sample using SERS **312**; comparing the determined amount of target analyte captured from the breath sample to a threshold level for the analyte in breath **314**; and indicating whether or not the determined amount of target analyte captured from the breath sample exceeds the threshold **316**. In some instance, the captured breath sample can be present on a SERS-active substrate having one or more capture sites (e.g., as described herein).

A captured breath sample can be analyzed or stored for later analysis. As seen in FIGS. **3**C-**3**D, some embodiments include a method for capturing an exhaled breath sample in a manner that allows for SERS-based detection at any useful time (e.g., immediately after capture or later). FIG. **3**C shows a general flow chart for a non-limiting method that includes capturing an exhaled breath sample from a subject at a location, in which the exhaled breath sample includes aerosol droplets **321**; and retaining the captured aerosol droplets in the structure for analysis for an analyte in the captured aerosol droplets **323**, wherein the capturing and retaining for analysis are conducted at the location **325**. In particular embodiments, the aerosol droplets are captured by impaction in a structure configured to excite surface plasmons (e.g., localized surface plasmons). Such a structure can include a droplet trap, in which the impaction site within the droplet site also includes a surface configured to propagating or exciting surface plasmons for SERS-based analysis. For instance, the surface can include a SERS-active substrate, a metal structure, or a metal nanostructure.

FIG. **3**D shows another general flow chart for a non-limiting method that includes capturing an exhaled breath sample from a subject at a location, in which the exhaled breath sample includes aerosol droplets **331**; retaining the captured aerosol droplets in the structure for analysis for an analyte in the captured aerosol droplets **333**; and optionally determining an amount of the analyte the captured aerosol droplets in the structure using SERS **336**. The determining operation **336** can be conducted at the location (in which the

capture operation **331** is conducted) or at a different location (e.g., a centralized lab, a forensics lab, etc.).

In any embodiment herein, capturing the aerosolized droplets or liquid drops in a breath sample can include the use of a droplet trap, as described herein. In particular, the droplet trap includes the use of a material that facilitates trapping of aerosol drops. In one embodiment, the droplet trap includes a material, which is provided as a substrate having one or more channels. Of these channels, one or more can be configured to facilitate droplet capture through inertial impaction. Another channel can include at least one passage that provides optical access to the trapped aerosol drop. This passage can, e.g., facilitate delivery of the excitation radiation (e.g., a laser light) to the trapped aerosol drops, as well as collection of SERS signals emanating from the trapped aerosol drops after exposure to excitation radiation. Such a passage can include a free optical path or an optical element (e.g., one or more mirrors, lenses, filters, beam splitters, optical fibers, etc., that can optionally bend or direct radiation signals) to deliver the SERS signal(s) to a spectrometer and/or detector configured to measure or detect a Raman-based signal.

Droplet Traps

Before diving into the design of droplet traps, it is useful to describe the basic mechanism of how aerosol droplets can be captured by inertial impaction. This technique involves driving a stream of fluid (in this case, air from a breath sample) containing the droplets or particles of interest from the impaction port **401** towards a stationary surface of a substrate **402** (or impaction substrate) and through a fluidic passage **410**. When the gas streamlines encounter the stationary surface, they turn parallel to the surface (see FIG. **4**A). The aerosol or particle droplets, however, have a higher inertia due to their higher densities, and are unable to make the turn necessary to stay with the gas/jet streamlines **405**. As a result, they impact the stationary surface of the substrate **402** (e.g., a surface including a SERS-active substrate), where they adsorb onto the stationary surface and are captured at the capture site **403**.

The process of inertial impaction is characterized by a universal dimensionless number called the Stokes' number (St), given by:

$$St = \frac{\rho_p d_p^2 v}{9\mu D}$$

where ρ_p is the density of the aerosol droplet or particle, d_p is the diameter of the aerosol droplet, v is the linear velocity of the air stream through the impaction port perpendicular to the impaction surface of the substrate, μ is the viscosity of the gas stream, and D is the hydraulic diameter of the impaction nozzle/port. For a given Stokes number, there is a capture efficiency (η) which represents the probability or fraction of particles with the particular Stokes number, that will be captured by the impaction surface.

A plot of capture efficiency versus log(St) typically shows a characteristic sigmoidal behavior for capture efficiency as a function of St. A characteristic property of the relationship between capture efficiency and St is a sharp transition between low and high capture efficiencies. This allows for inertial impaction to act as a binary collector, with very low capture efficiency for St numbers below a certain value and very high capture efficiencies for St above a certain threshold value. If we keep all other parameters fixed except for d_p in the expression for St, then St operates only as a function of the aerosol or particle size, d_p. For different values of d_p, we obtain different St values, which translates to a particular capture efficiency. Because of the sharp transition in capture efficiency as a function of St, there is a sharp transition in capture efficiency as a function of particle size, or d_p. The sharp-transition occurs around a particle size called as the cut-off diameter. An inertial impactor may thus act as a sieve, trapping all droplets above the cut-off diameter, and allowing smaller droplets to pass through completely.

Droplet traps designed for inertial impaction may utilize this strong relationship between St and capture efficiency. Based on this "sieve" property, the trivial solution is to design the droplet trap to capture the smallest droplet size desired, but there are practical limitations; and there is a trade-off between cut-off size and other parameters.

Generally speaking, droplet traps may feature turns and bends to facilitate the capture of droplets of particular size ranges. In the context of a largely planar structure or substrate, such as a microfluidic plate that may be suitable for analyzing collected samples having very small volumes, one particular type of droplet trap may utilize a plurality of small impaction ports that are positioned on an outer major surface (e.g., one of the larger, flat surfaces) of the planar structure. The droplet trap can be configured to interact with a housing, a valve structure, or another intermediary structure, which in turn can be adapted to interface with, for example, a mouthpiece or saliva trap.

In practice, a test subject (person) may place their lips around the mouthpiece or saliva trap to form a generally airtight seal, and may then exhale therethrough and into a device (including into a cavity or plenum within the device). The device may then serve to distribute the air from a person's exhaled breath to the plurality of small impaction ports. Each impaction port may overlap be in fluidic communication with a SERS-active substrate.

Generally speaking, as seen in FIG. **4**A, the impaction port **401**, when rectangular or otherwise oblong in nature, may be oriented such that the "short" axis **401***a* of the impaction port **401** is aligned with a major axis **410***a* of the fluidic passage **410**, as well as with the longitudinal or "long" axis **401***b* of the impaction port **401** being perpendicular or transverse to this major axis **410***a*. Such an arrangement can create a thin "sheet" flow of breath sample, which a) constrains the flow paths that the breath sample may follow when traversing the 90° bend to smaller radiuses of curvature (which increase the likelihood that a larger droplet will not be able to make the turn and will impact the floor of the fluidic passage **410**) and b) reduces the chance of turbulent flow, which may interfere with efficient droplet capture.

In some embodiments, the impaction port can have a longitudinal axis **401***b* that is perpendicular to a major plane (along axis **402***a*) of a SERS-active substrate **402**. Furthermore, the impaction port can be configured to be in fluidic communication with the one or more capture sites **403** disposed on a surface of the SERS-active substrate.

FIG. **4**B shows yet another embodiment of a droplet trap. As can be seen, the droplet trap can include an impaction port **451** disposed within a housing or a substrate disposed within the housing. This impaction port **451** can provide fluidic communication to a further substrate **452**, which serves to provide one or more capture sites for aerosol drops **450**. Fluidically disposed between the impaction port **451** and the substrate **452** can be a fluidic passage **460**, which is connected to a vacuum, thereby establishing flow **455** from an inlet (e.g., from the mouthpiece) to an outlet (e.g., a vacuum).

The substrate **452** can be configured to facilitate capture of aerosol drops, as well as to allow for detection of analytes within the drops. Accordingly, the substrate can include an impactor substrate (or an impactor surface) in conjunction with a SERS-active substrate (or a SERS-active surface). The configuration of such substrates and surfaces can be such to provide effective droplet capture, which can provide both enhanced concentration of analytes and minimized distance between the analyte and the SERS-active surface to provide increased SERS signals.

FIG. **4**C shows a non-limiting substrate can include integrated structures, such as a substrate having an impactor substrate **482** disposed beneath the SERS-active substrate **483**. The SERS-active substrate can include any useful metal nanostructures described herein. In use, aerosol drops **480** are captured on the SERS-active substrate **483**. In particular embodiments, a calibrator substrate **484** (or calibrator surface) can be included as an internal calibrator for SERS signals. In one instance, the calibrator substrate can include a deposited surfactant layer, which can provide a calibration signal upon exposure to a laser light to provide a calibrated SERS signal. In one instance, the calibration signal is used to normalize a SERS-signal, thereby providing a normalized SERS-signal indicative of the presence of the analyte (without significant contribution by the presence of the surfactant present in the calibrator surface). Accordingly, the integrated substrate may include a detection region **490** configured to capture aerosol drops and facilitate detection of analyte(s) and a calibration region **492** configured to provide calibration signals.

The calibrator substrate (or calibrator surface) can include any molecules that are present in control breath samples but not present in analyte-positive samples (e.g., positive samples being those that possess an amount of analyte that exceeds a threshold level of the analyte in breath). Such molecules can include those present in aerosol drops, such as surfactants (e.g., one or more lipids, phospholipids, and proteins). Non-limiting surfactants can include dipalmitoyl phosphatidylcholine (DPPC), palmitoyl-oleoyl phosphatidylcholine (POPC), surfactant protein A (SP-A), mucin, cholesterol, and the like, as well as combinations thereof. In particular embodiments, the calibrator substrate can be composed of one or more layers of surfactants. Such layers can include monolayers, bilayers, multilayers, as well as any useful combinations or plurality of such layers. In one embodiment, the integrated substrate includes an impactor substrate, a SERS-active substrate disposed on a top surface of the impactor substrates, and one or more calibrator substrates (e.g., including a deposited DPPC monolayer, bilayer, or multilayer) disposed on a portion of a top surface of the SERS-active substrate.

The droplet trap may include one or more impaction ports located along a path of the gas/jet streamline. FIG. **5** depicts a cross-section view of a portion of a droplet trap. As can be seen, the impaction port **520** extends through a substrate **502** and intersects with the fluidic passage **508**. Breath sample that flows through the impaction port **520** may enter the fluidic passage **508** and then make a 90° turn (arrows are added to indicate the general flow directions of the breath sample). Smaller particles (droplets), indicated by smaller-sized circles with lighter shading, in the breath sample flow may successfully navigate the 90° turn, whereas larger particles/droplets, indicated by larger-sized circles with darker shading, will generally not be able to make the 90° turn and will impact the floor of the fluidic passage **508** and adsorb onto it, forming a trapped portion of sample **528**.

The fluidic passage can also be designed to include capture sites **503**, in which the location of such capture sites **503** can be configured to capture liquid drops, as well as to capture any analytes associated with such liquid drops. To facilitate detection of analytes by employing SERS, the capture site(s) can further include a SERS-active substrate, such as any described herein.

In some embodiments, the capturing of the aerosol droplets by impaction involves capturing of the droplets through a plurality of impaction ports that are fluidically connected in parallel.

Capture by impaction provides a versatile approach that is readily adaptable to the capture of analytes including THC in breath. Breath borne analytes have been found to exist primarily in a non-volatile state in aerosolized droplets formed in the deep lung. As a result, the capture target is aerosolized droplets that can be viewed as particles having a an aerodynamic behavior based almost entirely on their size and shape, rather than the particular chemical or other affinity properties of an analyte of interest, as would be the case for a volatile target species. Since the capture is primarily based on the size of the aerosol droplets in the exhaled breath sample, the capture device or apparatus may be configured in the same or similar manner to capture virtually any analyte, by impaction. Then, the detection methodology may be tailored to the particular analyte(s) of interest in the aerosolized droplets captured by impaction, as further described below.

The described methods, devices and systems also have the merit of high yield capture of the component of an exhaled breath sample containing the analytes of interest, namely the aerosolized droplets originating in the deep lung. By contrast, alternative prior or potential methods of detecting breath-borne analytes have relied on affinity methodologies optimized for collection of volatile species in breath, or collection of breath condensate samples. Affinity-based collection techniques have low yield since breath borne analytes have been found to primarily be in non-volatile form and so with limited to no availability for affinity-based collection. Further, affinity-based collection of analytes requires very specific chemical or immunological targeting of the species to be collected, which limits the generality of the approach. Breath condensate collection, on the other hand, while general, lacks the specificity of capture by impaction and so provides a sample this is much less concentrated in and focused on the analytes of interest. This is a substantial impediment when attempting to meaningfully and reliably detect and measure very small quantities of analyte, such as exist in breath.

Described methods, devices and systems have the merits of sample capture by impaction and/or in a point of care format.

Design of a droplet trap or a breath capture module (BCM) begins with considerations on source and form of the target analyte in breath. With the exception of volatile small molecules, all other small molecules and macromolecules are present in breath encapsulated in aerosolized liquid drops in the range of about 0.5 μm-10 μm. The composition of the liquid drops consists primarily of water with other macromolecules associated with the respiratory tract.

For aerosolized liquid droplet targets, a mechanism of capture based on inertial impaction may be particularly effective, and a BCM may be designed with channels incorporating turns and bends to facilitate droplet capture through inertial impaction, as discussed herein. A two-stage mechanical filtering system may be used in some implementations to help screen out droplets that are larger and/or

smaller than a desired size range of droplets. For example, a saliva trap, such as saliva traps used with blood alcohol sensors, may be placed upstream of an inertial impaction droplet trap to filter out droplets that are larger than the upper end of the desired size range, e.g., larger than 100 μm, and the inertial impaction droplet trap may then be used to filter out those smaller droplets that pass through the saliva trap but are larger than the lower end of the desired size range, e.g., larger than 0.1 μm. The droplets that are captured by the inertial impaction droplet trap may generally be of the desired size range and be analyzed in situ or ex situ, such as in a system configured to interface with a device including the droplet trap.

In addition to the channel geometry, substrate material used for the fabrication of droplet traps may be selected, in some implementations, based on the properties of the target of interest. For molecular capture, the substrate material may be chosen such that the target has an affinity for the surface of the material and is immobilized on that surface after contact. The material may also facilitate release of the target into solution during elution or assay steps. Generally, the material may be weakly hydrophilic for a hydrophilic target and weakly hydrophobic for a lipophilic target. In other embodiments, the material can facilitate enhanced SERS-based detection, such as by including one or more SERS-active substrates, metal structures, etc.

However, for various non-volatile aerosolized species discussed in this disclosure, the BCM material may be designed to capture liquid drops, the primary constituent of which is water. For hydrophilic targets, a weakly hydrophilic substrate will enable retention of liquid drops during capture while facilitating release of these drops during elution or reaction steps. For hydrophobic targets, a strongly hydrophilic surface will discourage the target from adhering to the surface during elution or reaction steps. Macromolecules, particularly large proteins, are amphiphilic, which means they consist of both hydrophobic and hydrophilic regions. A hydrophilic capture material is appropriate for these molecules as well. In some embodiments, metal structures (e.g., surfaces of metal structures) can be treated to provide hydrophobic and/or hydrophilic regions. Such treatments can include use of oxidation, acid treatment, silanization, and the like, such as described herein.

In addition to surface characteristics, e.g., hydrophobicity or hydrophilicity of the material used for the sample collection sites of the droplet trap, analysis systems or other structures that interface with the droplet trap may be designed to facilitate efficient retrieval of or access to collected samples.

Typical captured droplets from exhaled breath may include a high percentage (>50% by mass) of surfactants such as phospholipids, such as DPPC (dipalmitoyl phosphatidylcholine). These surfactants may include long-chain aliphatic carboxylic acids which render them highly lipophilic. When aerosol droplets are captured on a surface, due to the extremely low volumes (pL or less) of droplets, the water contained in these droplets can evaporate very quickly (especially considering the flow of exhaled air that flows past them during droplet capture), resulting in a concentrated patch or "scab" of lipophilic surfactants on the surface(s) of the capture sites in the droplet trap. It is within these "scabs" that the analytes of interest may be trapped.

As discussed above, surface modifications, e.g., surface treatments to render the surfaces on which the "scabs" form more or less hydrophilic or hydrophobic, may be used to increase or enhance the recovery of collected sample material.

Surface modification may be used to create a surface which prevents phospholipids from forming a "scab" on the surface of the droplet trap (or at least from forming a "scab" that is strongly adhered to that surface). Potential modifications include coating the surface with polymers such as tri-block copolymers containing repeating ethylene oxide and propylene oxide groups or biological macromolecules such as proteins (examples include an albumin, such as bovine serum albumin (BSA), casein, etc.). In these two cases, the mechanism of deposition of the surface treatment may be physical adsorption, wherein the coating agent is allowed to incubate with, for example, a surface of the SERS-active substrate having the capture sites, and the agent is allowed to adsorb onto the surface, forming a barrier. Alternately, the surface can be treated chemically to impart specific functionality. Silanizing is one such method of surface treatment. In this process, a silanizing agent such as trichloro silanol may be allowed to react with the surface (or alternately air) to form a silanized coating on the surface. The silanized coating can create a barrier between the phospholipids and the plastic surface.

In some cases, the analyte may be present in alveolar lining fluid (ALF) which is present in exhaled breath as aerosolized droplets, for which a geometry (of the droplet trap) based on inertial impaction can produce high capture efficiency. A hydrophilic material may be used for capturing these droplets. Suitable materials for such a droplet trap may include polystyrene, polyethylene terephthalate (PET), PETG (glycol modified version of polyethylene terephthalate), glass, etc. A droplet trap with a hydrophilic material (treated polystyrene, PETG, glass, etc.) in a geometry designed for inertial impaction may provide good capture of droplets/particles of interest.

A droplet trap for capture aerosol drops via inertial impaction may be designed, according to the principles and concepts outlined herein, to intercept aerosol particles >0.7 μm, for example, and retain them on the walls of a channel through which breath is flowed while the droplet trap is used to collect a breath sample. Multiple impaction sites may be incorporated into the droplet trap to provide for parallel capture of droplets, thereby allowing for higher droplet capture efficiency and greater breath throughput.

In addition to flow considerations, a handheld design may, in some instances, also facilitate specific breathing profiles such as rapid inhalation, rapid exhalation, coughing, etc. to stimulate production of volatiles or aerosol drops from appropriate regions of the respiratory tract. This can be accomplished, for example, using sensors such as pressure, flow rate, and CO_2 sensors to monitor breath sample collection.

Fluidic communication, as the phrase is used herein, refers to a state in which two or more volumes are connected by one or more passages, orifices, or other features such that fluid may flow between them. Generally speaking, the phrase should be understood to imply that there is some form of structure providing the fluidic communication, rather than just exposure to the ambient environment. For example, two open-topped buckets positioned side-by-side in upright positions would not be considered to be in "fluidic communication" (even though fluid, e.g., gas, could conceivably waft of diffuse from one bucket to the other), whereas placing an end of a hose into each of those same two open-topped buckets would cause the buckets to be viewed as being in "fluidic communication" with each other since there is structure that serves to provide a fluid flow passage between them.

SERS-Based Detection

Surface-Enhanced Raman Spectroscopy (SERS) combines Raman scattering and Surface Plasmon Resonance (SPR). SERS uses SPR to enhance surface sensitivity of Raman scattering resulting in a large enhancement of a Raman scattered signal. For example, SERS results in a 10^3 to 10^{10}-fold signal increase compared to traditional "bulk" Raman scattering. The present technology provides systems and methods using SERS for detecting analytes (e.g., cannabinoids including tetrahydrocannabinol (THC)) for label-free THC detection in breath samples. The present technology in some embodiments provides systems and methods using SERS for detecting THC with single molecule detection sensitivity. The systems, methods and contemplated devices of the present technology may also be adaptable to combining testing for THC and alcohol (ethanol) impairment, and/or to the detection of other airborne substances, including controlled substances, and breath-borne indicators of various disease states and viruses.

Raman scattering or the Raman effect is the inelastic scattering of input electromagnetic radiation (e.g., photons) by matter in various embodiments. Such inelastic scattering is dependent on the type of matter or molecule with which it interacts, such that the Raman scattering signal (output) can be used to gain information about that matter or molecule. Stokes Raman scattering arises from vibrational energy being gained by a molecule as incident photons are shifted to a lower energy.

Furthermore, Raman scattering requires a change in polarizability, and allowable Raman transition states require different molecular polarizability of those states. A polarized molecule will oscillate at the same frequency as an input electric field (E) of an electromagnetic wave:

$$P=\varepsilon_0\chi E,$$

wherein P is the polarization density, ε_0 is the electric permittivity in vacuum, and χ is the electric susceptibility.

Molecules also have vibrational modes. Electric susceptibility will oscillate at the vibrational energy modes of a molecule. Thus, an induced dipole moment will be modulated by vibrational oscillations of the molecule:

$$P = \underbrace{\alpha_0 E_0 \cos(2\pi\nu_0 t)}_{\text{Rayleigh scattering}} + \left(\frac{\partial\alpha}{\partial Q}\frac{Q_0 E_0}{2}\right)\left\{\underbrace{\cos[2\pi(\nu_0-\nu_{vib})t]}_{\text{Stokes scattering}} + \underbrace{\cos[2\pi(\nu_0-\nu_{vib})t]}_{\text{anti-Stokes scattering}}\right\},$$

wherein α is the polarizability of the molecule, α_0 is the polarizability at equilibrium, E_0 is the amplitude of the electromagnetic wave, ν_0 is the frequency of the electromagnetic wave, ν_{vib} is the vibrational frequency of the molecule, Q is a bond length at any instant, and Q_0 is a maximum displacement distance of atoms relative to their equilibrium position.

Raman scattering is conceptualized as involving a virtual electronic energy level, which corresponds to the energy of the exciting laser photons. Absorption of a photon excites the molecule to the imaginary state, and re-emission leads to Raman or Rayleigh scattering. In all three cases, the final state has the same electronic energy as the starting state but is higher in vibrational energy in the case of Stokes Raman scattering, lower in the case of anti-Stokes Raman scattering, or the same in the case of Rayleigh scattering. FIG. **6** provides a basic Quantum Mechanics (QM) description of Raman scattering, which includes a photon exciting the molecule to the imaginary state for Stokes Raman scattering, Rayleigh scattering, and anti-Stokes Raman scattering.

The following illustrates an equation for a Raman signal, according to various embodiments of the present technology:

$$I\propto I_0 N\sigma,$$

wherein I is the intensity of the Raman signal, I_0 is the incident optical irradiance [w cm^{-2}], N is the number of molecules, and σ is the Raman scattering cross section. As can be seen, the Raman signal is proportional to concentration of a molecule to be detected (i.e., number of molecules). For example, a Raman signal can be proportional to the number of molecules of THC in a breath sample. Other factors that can influence the intensity for a Raman signal include excitation/collection optics efficiency, background signals, and the like.

FIG. **7** illustrates a review of Surface Plasmon Resonance (SPR), according to various embodiments of the present technology. SPR is the resonant oscillation of conduction electrons at the interface between negative and positive permittivity material stimulated by incident light. SPR is a fundamental principle behind embodiments of the present technology and is the basis of many tools for measuring adsorption of material onto planar metal (e.g., typically gold or silver) surfaces or onto the surface of metal nanoparticles (e.g., gold or silver nanoparticles). Electromagnetic (EM) waves incident at a metal-dielectric boundary can create a coherent, surface wave having a wavelength λ_{SPP}, as shown in FIG. **7**. For example, electron oscillation at the surface, as a result of the interaction between light and free electrons, can propagate a surface wave at the metal-dielectric interface along axis x.

SPR only exists when certain requirements are met at an interface of two materials. In one instance, a specific combination of dielectric constants (ε) can be provided, such as between a metal having ε_1 and a dielectric material having ε_2 that is a positive real dielectric constant (and having no imaginary part, such as for air or insulating materials). In another instance, the interface is characterized by having negligible bulk effects, such as can be present by a sufficiently thin layer (along axis z) that can sufficiently propagate a surface wave with negligible influence by a bulk material disposed beneath a surface layer. Furthermore, an EM wave must decay in a direction normal to surface (i.e., as an evanescent wave).

The characteristics of SPR can be highly dependent on other factors, such as the following: dielectric properties of the materials at the interface; wavelength, angle and polarization of excitation; a thickness of the metal layer (e.g., bulk effects may hinder SPR); and/or the roughness of the surface on the order of nanometer scale.

Resonance condition occurs at the right wavelength and angle. In various embodiments, the resonance condition is characterized by a strong absorption of light at the right excitation wavelength and at the right excitation angle. Furthermore, a small change in dielectric properties of the insulating medium may result in a large signal change. Moreover, SPR has a high sensitivity to changes at the interface (e.g., due to binding of an analyte such as THC). In one instance, such changes at the interface can provide a detectable shift in wavelength of the resonance peak.

Surface-Enhanced Raman Spectroscopy (SERS) generally employs localized surface plasmon resonance (LSPR) to generate enhanced Raman signals. FIG. **8**A-**8**B shows the effect of using SERS, according to various embodiments of the present technology. In FIG. **8**A, a weaker Raman scat-

tering **806** is shown, as compared with a stronger Raman scattering **816** shown in FIG. **8**B. For instance, in FIG. **8**A, the analyte **801** is disposed on a planar surface of a substrate **802**, in which the excitation radiation **805** provides weak Raman scattering **806** in conjunction with Rayleigh scattering **807**.

In contrast, FIG. **8**B provides an analyte **811** disposed on a surface of a substrate **812** having metal structures **813** (e.g., metal nanostructures), in which the excitation radiation **815** provides stronger Raman scattering **816** in conjunction with Rayleigh scattering **817**. If a Raman active molecule **811** (e.g., THC) is present in the vicinity of Localized Surface Plasmon Resonance (LSPR) and concentrated light **814**, then strong EM wave coupling can enhance the Raman signals. Raman wavelengths must be close to the SPR wavelength for SERS, and the molecule must be within the decay distance of the electromagnetic enhancement. The enhancement factor depends on dielectric properties, as well as geometry. The enhancement factor can be influence by both EM wave enhancement and chemical enhancement for SERS. See, e.g., Vahimaa P et al., "*Surface-Enhanced Raman Spectroscopy (SERS),*" Institute of Photonics at the University of Eastern Finland, accessible at sway.com/s/XtgAoh8F5QewSEFL/embed, which is incorporated herein by reference in its entirety.

FIG. **9** illustrates an example of enhanced sensitivity of Surface-Enhanced Raman Spectroscopy (SERS), as compared with "bulk" Raman scattering, according to various embodiments of the present technology. In FIG. **9**, Raman shift vs. intensity is shown comparing a SERS method with a non-SERS method for rhodamine 6G that was excited at 633 nm with a 3 mW incident laser. In this non-limiting example, SERS provided single molecule sensitivity, as compared with 7.8×10^5 molecules for non-SERS methods, e.g., see E. C. Le Ru E C et al., "*Surface Enhanced Raman Scattering Enhancement Factors: A Comprehensive Study,*" *J. Phys. Chem. C,* 111, 13794-13803 (2007) (hereinafter "Le Ru et al."), which is incorporated herein by reference in its entirety; and in which FIG. **1**, FIG. **S1**, and associated text in Le Ru et al. are incorporated herein by reference, especially as it relates to SERS and non-SERS Raman signals, determination of differential cross-sections, assessment of single molecule (SM) SERS events, and analysis of SM enhancement factor (SMEF).

FIG. **10** illustrates a spherical nanoparticle model for SERS, according to various embodiments of the present technology. As can be seen, the LSPR can be characterized by a collective oscillation of valence electrons (electron cloud **1002**) for a metal nanoparticle **1001** that is in resonance with the frequency of incident light **1005**. The resultant electromagnetic field **1006** outside the particle has an analytical solution, in which maximum enhancement of the SERS signal **1007** occurs under certain conditions (e.g., $\varepsilon_1\approx-2\varepsilon_2$). In another embodiment, the extent of SERS enhancement leading to single molecule sensitivity is due to a variety of factors such as nanoparticle size (e.g., having a radius a), shape, material (e.g., having a dielectric constant ε_1), configuration, and the like. See, e.g., Stiles P L et al, "*Surface-Enhanced Raman Spectroscopy,*" *Annu. Rev. Anal. Chem.,* 1, 601-626 (2008) (hereinafter "Stiles et al."), which is incorporated herein by reference in its entirety; in which FIG. **1** and associated text in Stiles et al. are incorporated herein by reference, especially as it pertains to the discussion regarding localized surface plasmon resonance; in which FIG. **2** and associated text in Stiles et al. are incorporated herein by reference, especially as it pertains to the discussion regarding E^4 enhancement; and in which FIG. **4** and associated text in Stiles et al. are incorporated herein by reference, especially as it pertains to the discussion regarding instrumentation, nanofabrication, and optimized surface-enhanced Raman spectroscopy surfaces.

As discussed herein, metal materials are of particular use in SERS-active surfaces, and the dielectric constant of such materials can affect the extent of maximizing the enhancement factor (EF). In one instance, EF can be maximized by maximizing g:

$$g=\frac{\varepsilon_1-\varepsilon_2}{(\varepsilon_1+2\varepsilon_2)},$$

wherein ε_1 is the dielectric constant for the metal particle and ε_2 is the dielectric constant of the external environment. Using the equation above, g can be maximized by having $\varepsilon_1\approx-2\varepsilon_2$. Assuming that a particular breath sample includes DPPC in the external environment in conjunction with the target analyte, the dielectric constant of DPPC can be considered as ε_2. In one non-limiting consideration, ε_2 for DPPC can be between 2 to 4.5 (e.g., from 2 to 4, 2.2 to 4.2, 3 to 3.5, or about 3.2). See, e.g., Gramse G et al, "*Nanoscale Measurement of the Dielectric Constant of Supported Lipid Bilayers in Aqueous Solutions with Electrostatic Force Microscopy,*" *Biophys. J.,* 104, 1257-1262 (2013) (hereinafter "Gramse et al."), which is incorporated herein by reference in its entirety; and in which FIG. **4** and associated text in Gramse et al. are incorporated herein by reference, especially as it relates to experimental capacitance gradient approach curves and fitting parameters to characterize such data, including parameters such as the dielectric constant for the lipid bilayer (e.g., a dielectric constant $\varepsilon_{r,DPPC}$ of about 3.2).

By assuming a value (or range of values) for ε_2, a metal material can be selected under conditions to provide a dielectric constant for the metal (ε_1) that is approximately $-2\varepsilon_2$. The dielectric constant for a metal can be dependent on the irradiation wavelength or photon energy, and the dielectric constant (as a function of wavelength and composed of real and imaginary parts) can be characterized in any useful manner. For instance, the dielectric function of gold (Au) in the visible spectral region can be characterized by spectroscopic ellipsometry measurements or other useful measurements, see, e.g., Olmon R L et al., "*Optical dielectric function of gold,*" *Phys. Rev. B* 86, 235147 (2012) (hereinafter "Olmon et al."), which is incorporated herein by reference in its entirety; and in which FIG. **3** and associated text in Olmon et al. are incorporated herein by reference, especially as it relates to the dielectric function of Au (the negative real part $-\varepsilon1$) in the visible spectral region for evaporated (EV), template-stripped (TS), and single-crystal (SC) gold samples, and as it relates to variables that can be determined by fitting the data in FIG. **3** to a solution of the Drude-Sommerfeld model or to a Drude dielectric function; and in which FIG. **4** and associated text in Olmon et al. are incorporated herein by reference, especially as it relates to the dielectric function of Au (the imaginary part ε_2) in the visible spectral region for EV, TS, and SC gold samples, and as it relates to variables that can be determined by fitting the data in FIG. **4** to a solution of the Drude-Sommerfeld model or to a Drude dielectric function.

In another instance, the dielectric function of silver (Ag) in the visible spectral region can be characterized by spectroscopic ellipsometry measurements or other useful measurements, see, e.g., including the negative real part and the

imaginary part, according to various embodiments of the present technology. See Yang H U et al, "*Optical dielectric function of silver,*" *Phys. Rev. B* 91, 235137 (2015) (hereinafter "Yang et al."), which is incorporated herein by reference in its entirety; and in which FIG. **3** and associated text in Yang et al. are incorporated herein by reference, especially as it relates to the negative real part of the dielectric function of silver (−ε1) in the visible/ultraviolet spectral range for template stripped (TS) silver samples, and as it relates to variables that can be determined by fitting the data in FIG. **3** to a solution of the Drude-Sommerfeld model or to a Drude dielectric function; and in which FIG. **4** and associated text in Yang et al. are incorporated herein by reference, especially as it relates to the imaginary part of the dielectric function of silver (ε2) in the visible/ultraviolet spectral range for TS samples, and as it relates to variables that can be determined by fitting the data in FIG. **4** to a solution of the Drude-Sommerfeld model or to a Drude dielectric function.

By assuming a value (or range of values) for ε_2 for the external environment, a metal material can be selected under conditions to provide a dielectric constant for the metal (ε_1) that is approximately $-2\varepsilon_2$. In one embodiment, the external environment in proximity to the analyte is considered to be DPPC, the metal material includes gold, and the irradiation wavelength is selected to be from about 500-650 nm (e.g., from 500-600 nm, 550-650 nm, or about 600 nm). In some embodiments, the EF is from about 10^3-10^6. In another embodiment, the external environment in proximity to the analyte is considered to be DPPC, the metal material includes silver, and the irradiation wavelength is selected to be from about 400-550 nm (e.g., from 400-500 nm, 450-500 nm, or about 488 nm). In yet other embodiment, the EF is from about 10^5-10^8.

The equation below illustrates the distance dependence for SERS, in which the SERS signal is maximized when the target analyte is absorbed to the enhancing surface, according to various embodiments of the present technology:

$$I_{SERS} = \left(\frac{a+r}{a}\right)^{-10},$$

wherein a is the average size of the field-enhancing features on the SERS-active substrate and r is the distance from the surface to the adsorbed analyte, see, e.g., Stiles et al., which is incorporated herein by reference, and in which section 2.3 in Stiles et al. is incorporated herein by reference, especially as it relates to the distance dependence of SERS.

FIG. **11**A-**11**B illustrates exemplary systems and instrumentation for SERS, according to various embodiments of the present technology. FIG. **11**A-**11**B illustrates two instrumental approaches to the measurement of SER spectra. The first approach shown in FIG. **11**A is for a non-limiting macro-Raman configuration. Here, a laser source **1102** is focused on the SERS-active substrate having the sample **1101** at a glancing angle by way of a mirror M1 **1104**. After irradiation, the resulting SERS signal (Raman signal) light is collected by way of a lens L1 **1106** (e.g., a collection lens). The light can then be filtered by way of filter F1 **1108** (e.g., a notch filter) and delivered to the entrance slit of a spectrometer **1110** and detected using a detector **1112**, e.g., a liquid-nitrogen-cooled charged-coupled device camera. The instrumental approach shown in FIG. **11**A is, in some instances, used in low spatial resolution and high raw SERS intensity. Nonetheless, such a configuration can be adapted for high resolution and/or low raw SERS intensity signals.

Any optical path herein (e.g., from a laser source to the sample and/or from the sample to the spectrometer or detector) can include any useful optical element to direct, split, focus, filter, and/or process a radiative signal. Non-limiting optical elements include one or more mirrors, fibers, splitters, lenses, filters, objectives, and the like, which can include mechanical or electronic forms thereof. In a particular instance, the optical path can include use of optical fiber, an optical probe, or other elements, which can include miniaturized configurations for use with a handheld apparatus. In some instances, a spectrometer, a detector, or both can be employed.

For higher spatial resolution, a micro-Raman configuration can be used, e.g., as shown in FIG. **11**B. Irradiation (or laser light) from a laser source **1152** can be directed to the sample **1151** by way of a mirror M1 **1154**, in which the light is both focused and collected through the same high numerical aperture objective O1 **1155** before delivery to the sample **1151**. After, the scattered light is passed through a filter F1 **1158** (e.g., a notch filter) for the removal of Rayleigh-scattered light. A beam splitter S1 **1153** can be employed between the laser source **1152** and the spectrometer **1160**. Additionally, the light can be focused by a lens L1 **1156** and directed to a spectrometer **1160** and/or a detector **1162**. See, e.g., Stiles et al., which is incorporated herein by reference in its entirety; and in which FIG. **4** and associated text in Stiles et al. are incorporated herein by reference, especially as it pertains to the discussion regarding instrumentation.

SERS can be employed for detection of THC molecules, according to various embodiments of the present technology. In one instance, SERS-based detection can be employed to provide spectra of THC molecules (at various concentration, e.g., 10^{-3}, 10^{-5}, 10^{-7}, 10^{-9}, and 10^{-12} M) on a SERS-active substrate, in which the SERS interface is gold and air. In one embodiment, the intensity of the SERS signal (I_{SERS}) at 1603 cm^{-1} is correlated to a concentration of THC in the sample. In another embodiment, the SERS signal is obtained from a range of 1500-1700 cm^{-1}, in which the signal in this range is correlated to a concentration of THC in the sample. In yet other embodiments, the SERS signal is obtained from at least one of about 1000, 1030, 1195, 1201, 1275, 1279, 1283, 1323, 1370, 1377, 1382, 1405, 1450, 1546, 1556, 1580, 1595, 1603, 1621, and 1651 cm^{-1}. See, e.g., Sivashanmugan K et al., "*Trace Detection of Tetrahydrocannabinol in Body Fluid via Surface-Enhanced Raman Scattering and Principal Component Analysis,*" *ACS Sensors,* 4, 1109-1117 (2019) (hereinafter "Sivashanmugan et al."), which is incorporated herein by reference in its entirety; and in which FIGS. **2**A-**2**B and associated text in Sivashanmugan et al. are incorporated herein by reference, especially as it relates to the SERS spectra of THC molecules on a plasmonic-biosilica SERS substrate and to associated SERS peaks at 1000, 1030, 1201, 1275, 1323, 1370, 1450, 1556, 1580, and 1603 cm^{-1}; and in which FIG. **3**A-**3**D, FIGS. **4**A-**4**D, and associated text in Sivashanmugan et al. are incorporated herein by reference, especially as it relates to the SERS spectra of THC molecules in complex fluids on a plasmonic-biosilica SERS substrate and to associated SERS peaks at 1195, 1279, 1283, 1323, 1377, 1382, 1405, 1546, 1595, 1603, 1621, and 1651 cm^{-1}.

In various embodiments, a SERS-active substrate includes or is Ag nanoparticles in diatom photonic biosilica. Furthermore, the sample may be dried onto substrate, and detection having a sensitivity to about 1 pM has been demonstrated. Furthermore, different solvents (e.g., metha-

nol, plasma, saliva, alveolar fluid, lung epithelial fluid, lung aspirate, aerosol drops, surfactants, water, and the like, as well as combinations thereof) may be used.

The apparatuses and devices herein can be employed with a system for using SERS for detection of THC molecules, according to various embodiments of the present technology. In one embodiment, the exemplary system can include a high sensitivity spectrometer, an excitation laser or diode source (e.g., a 670 nm excitation source, a 600 nm excitation source, a 488 nm laser excitation source, or any other excitation source of any wavelength described herein), a Raman probe (e.g., optic Raman probe), and/or a detector. Such systems can include any other useful optical elements to optically access the sample, the SERS-active substrate, or a portion thereof. Optical elements can include one or more mirrors, lenses, fibers, filters, wave plates, as well as any described herein.

SERS-active substrates include those having any material that can sustain and propagate surface plasmons, including localized surface plasmons. In one instance, the material include nanostructured metal, including metal nanoparticles (e.g., core-shell particles, solid particles, etc.), nanowires, nanotubes, nanoantennas, nanogratings, nanopillars, nanowrinkles, nanorods, nanocapsules, nanospheres, nanoprisms, nanocubes, nanostars, and nanosheets. See, e.g., Wang A X et al., "*Review of Recent Progress of Plasmonic Materials and Nano-Structures for Surface-Enhanced Raman Scattering," Materials,* 8, 3024-3052 (2015) (hereinafter "Wang et al."), which is incorporated herein by reference in its entirety; and in which FIGS. **4**A-**4**D and associated text in Wang et al. are incorporated herein by reference, especially as it relates to plasmonic nano-structures and metallic nanomaterials with different geometric morphologies. A nanostructure can include any structure having a feature size (e.g., length, width, height, radius, diameter, circumference, gap, periodic distance, etc.) that is from about 0.1-100 nm.

Such nanostructures can be provided in any useful assembly, e.g., as a monolayer, a packed layer, a packed volume, a sheet, a colloid, a colloidal crystal, etc. Such nanostructures can be disposed on a surface of a substrate in any useful manner, such as by patterning, deposition, growth, laser treatment, drilling, lithography, etc. Non-limiting substrates can include a semiconductor material (e.g., silicon or a dielectric, such as silicon nitride), graphene, glass, diatoms, and the like. In one instance, the nanostructures provide a nanostructured surface. In various embodiments multiple types of surfaces are used for SERS including nanoparticles, "roughened" thin films by laser ablation, nanoholes, nanospheres, and nanowires. Each of the surface types used for SERS produces a different enhancement factor, as well as efficiency of capturing the sample. Furthermore, other practicality factors include reproducibility, limitation of laser excitation power, fluorescence, and Raman background from the surface, as well as the shelf-life and environmental stability.

FIG. **12**A-**12**C illustrates an exemplary breath capturing device using SERS for detecting trace levels of molecules including cannabinoids, such as THC in breath samples, according to various embodiments of the present technology. FIG. **12**A shows a subject being tested using a breath capture device **1201** by exhaling through a mouthpiece **1202**.

FIG. **12**B also shows a non-limiting cross-sectional view of breath capturing device. In some embodiments, a breath sample goes inside the breath capturing device by way of a subject breathing through a mouthpiece **1212**, which can be a portion of the housing or be a separate structure (e.g., a separate detachable structure). Inside of the housing **1211**, the device comprises breath sample capture sites on a SERS-active substrate. Each of portions **1213***a/b/c/d* can each be a SERS-active substrate, such that the device includes a plurality of SERS-active substrates. Alternatively, each of portions **1213***a/b/c/d* can each be a capture site, in which capture sites **1213***a/b/c/d* can be disposed on a single SERS-active substrate or different SERS-active substrates. For example, local breath sample capture sites can be disposed on a surface of the SERS-active substrate. The substrate may comprise metal nanoparticles, a nanoparticulate metal surface (e.g., surface of gold or silver nanoparticles), or any other nanostructured metal described herein. The location of each capture site and/or SERS-active substrate can be configured to optimally capture a breath sample, aerosol drops from a breath sample, and the like (e.g., by way of impaction). In particular embodiments, the capture site and/or SERS-active substrate are configured to be easily removed from the housing, thereby facilitating ex situ analysis or storage for later analysis.

In particular embodiments, the SERS substrate causes localized surface plasmons to be excited, greatly enhancing Raman signals. The enhancement can be significant, making SERS capable of trace level detection of molecules allowing using SERS for detecting THC in breath samples with single molecule detection sensitivity. The device can also comprises an optical detection device **1210** (e.g., a spectrometer or detector configured to optically access the capture sites(s) and/or SERS-active substrate(s)) or an interface **1210** configured to optically couple to a detection device (e.g., in which the interface allows for a spectrometer or detector configured to optically access the capture sites(s) and/or SERS-active substrate(s)).

Optical coupling or optical access, as the phrase is used herein, refers to a state in which two or more areas or volumes are connected by an optical path or one or more optical elements (e.g., any herein) or other features such that an optical signal (e.g., light) may travel between them. This phrase should be understood to imply that one or more structures may be present to provide optical coupling or optical access or that an ambient environment (e.g., air) that facilitates travel of light may also be envisioned.

FIG. **12**C shows a cross-sectional view of another non-limiting breath capturing device. The device includes a housing **1221** and a mouthpiece **1222**, which can be a portion of the housing or be a separate structure (e.g., a separate detachable structure). Inside of the housing **1221**, the device comprises breath sample capture sites on a SERS-active substrate. Each of portions **1223***a/b/c/d* can each be a SERS-active substrate, such that the device includes a plurality of SERS-active substrates. Alternatively, each of portions **1223***a/b/c/d* can each be a capture site, in which capture sites **1223***a/b/c/d* can be disposed on a single SERS-active substrate or different SERS-active substrates.

The device can further include a plate **1225** or another structure having impaction ports **1226** disposed therein. Such a plate (or another structure) can be disposed within the main volume within the housing **1221** or within the narrower volume within the mouthpiece **1222**. As can be seen, one or more of the impaction ports **1226** can serve to direct the fluid flow, such that the breath sample is captured by way of impaction on a capture site/SERS-active substrate **1223***a/b/c/d*. The location of each capture site/SERS-active substrate **1223***a/b/c/d* within the housing **1221**, from the impaction ports **1226**, from the mouthpiece **1222**, etc., can be optimized to provide effective droplet separation and/or droplet capture.

The device can also comprises an optical detection device **1220** or an interface **1220** configured to optically couple to a detection device, as well as one or more optical elements **1228** to provide optical access to the capture site/SERS-active substrate **1223***a/b/c/d*. Non-limiting optical elements are described herein.

The devices and apparatuses herein can be employed within a breath sampling and analysis system. In one instance, the system can include three main components—a base station, a handheld unit, and a cartridge (e.g., a disposable cartridge). In another instance, the system can include two main components—a base station and a handheld unit. In yet another instance, the system only includes a handheld unit.

Furthermore, when a cartridge is present, a handheld unit may be connected with the cartridge in order to collect a breath sample, as the handheld unit can be small, relatively lightweight, and easily wielded by whomever is obtaining the breath sample. The cartridge may then be removed from the handheld unit, and both elements can be separately docked in the base station in order to perform the analysis and report out the results. The cartridge can include the captured sample disposed on the capture sites and the SERS-active substrate. While the functionality of the cartridge could be combined with a handheld unit, although doing so may complicate cleaning and re-use of the handheld unit.

Optionally, the handheld unit can include an integrated SERS-active substrate including the capture sites, such that a cartridge is not required. In this embodiment, the handheld unit can then be docked into the base station for analysis, in which the optical interface of the handheld unit is optically coupled to the base station. Alternatively, the handheld unit can include an integrated detector. For example, the base station and the handheld unit could be combined into one device, although the resulting apparatus would not be as portable as the handheld unit and obtaining a breath sample using such an apparatus would likely require extra effort on behalf of the subject.

While the breath sampling and analysis system discussed herein as an example is designed for use as a THC detection system, it will be understood that similar systems, with appropriate modification, may be used to detect one or more additional or alternative analytes, as noted earlier. For example, the breath sampling and analysis system architecture discussed herein may also be used generally to capture breath samples that may then be analyzed to determine amounts of other controlled substances (or byproducts of using such controlled substances). In general, the systems and architecture provided herein allow for breath samples containing potentially very small volumetric densities or concentrations of analytes, e.g., with magnitudes on the picogram-per-liter scale, to be captured and concentrated in reaction volumes on the order of microliters or tens of microliters within microfluidic circuits/plates. Once captured, such volumes may be analyzed to determine the presence and quantity of a particular analyte of interest, e.g., according to any of the assay techniques discussed earlier. As discussed, while most of the discussion herein is with reference to an example such system for detecting THC, the principles set forth herein, and the overall architecture, may be applicable to systems for detecting a variety of different analytes, and the concepts laid out herein should not be viewed as being solely directed to THC detection systems and methods.

According to various embodiments the present technology provides a simplified design using label-free, direct detection of THC in breath samples (i.e., rather than urine, saliva, or blood samples) using a SERS substrate. Furthermore, SERS produces Raman signals that are orders of magnitude greater than traditional Raman scattering detection ("bulk Raman"), thus, the present technology allows enhanced sensitivity including single molecule sensitivity of THC in breath samples. Moreover, the present technology allows specific detection of THC because the SERS spectrum is a "signature" of the molecule being detected rather than using a label. Additionally, since measurement is done right at the point of collection in various embodiments, the target analyte (e.g., THC) is in its most concentrated form, allowing for maximal use of a collected sample (e.g., breath sample).

Data obtained from any of the devices, apparatuses, systems, and methods herein can be retained within the device in which the measurement has been made. Further, such data may be otherwise stored (e.g., in a memory, a server, a cloud server, etc.), transmitted (e.g., in a wired or wireless manner to a local network or the Internet, etc.), or communicated in any useful manner.

OTHER EMBODIMENTS

This application incorporates by reference the following applications for their disclosure relating to implementation of biomarker (which may be considered an analyte) capture, collection, detection, measurement and analysis methods and apparatus that are suitable for implementation of the disclosed methods and devices: U.S. application Ser. No. 16/425,938, filed May 29, 2019, titled "SINGLE-USE MICROFLUIDIC CARTRIDGE FOR DETECTION OF TARGET CHEMICAL PRESENCE IN HUMAN BREATH" and U.S. application Ser. No. 16/425,943, filed May 29, 2019, titled "MECHANICAL BREATH COLLECTION DEVICE" and International Application No. PCT/US2020/013553, filed Jan. 14, 2020, published as International Publication No. WO 2020/159698, titled "MECHANICAL BREATH COLLECTION DEVICE" and U.S. application Ser. No. 16/776,501, filed Jan. 29, 2020, titled "NONINVASIVE POINT OF CARE BIOMARKER DETECTION FROM BREATH SAMPLES," each of which claims priority to U.S. Provisional Patent Application No. 62/799,675, filed Jan. 31, 2019, titled "NON-INVASIVE POINT OF CARE BIOMARKER DETECTION FROM BREATH SAMPLES"; U.S. application Ser. No. 16/729,116, filed Dec. 27, 2019, titled "ANALYTE DETECTION FROM BREATH SAMPLES," which claims priority to U.S. Provisional Patent Application No. 62/786,222, filed Dec. 28, 2018, titled "ANALYTE DETECTION FROM BREATH SAMPLES"; U.S. application Ser. No. 16/823,113, filed Mar. 18, 2020, titled "BIOMARKER DETECTION FROM BREATH SAMPLES," which claims priority to U.S. Provisional Application No. 62/821,900, filed Mar. 21, 2019, titled "BIOMARKER DETECTION FROM BREATH SAMPLES"; from U.S. application Ser. No. 16/124,181, filed Sep. 6, 2018, titled "ANALYTE DETECTION FROM BREATH SAMPLES," which claims priority to U.S. Provisional Application No. 62/646,798, filed Mar. 22, 2018, titled "ANALYTE DETECTION FROM BREATH SAMPLES"; U.S. application Ser. No. 16/655,182, filed Oct. 19, 2019, titled "ROTARY VALVE ASSEMBLIES AND METHODS OF USE FOR BREATH SAMPLE CARTRIDGE SYSTEMS," which claims prior to U.S. Provisional Application No. 62/746,858, filed Oct. 17, 2018, titled "BREATH SAMPLE CARTRIDGE AND SYSTEM"; and U.S. Provisional Application No. 63/201,389,

filed Apr. 27, 20201, titled "BREATH ANALYTE DETECTION AND MEASUREMENT."

This application also incorporates by reference the following applications for their disclosure relating to implementation of biomarker (or analyte) collection and detection methods and apparatus that are suitable for implementation of the disclosed methods and devices: U.S. Provisional Application No. 62/557,056, filed Sep. 11, 2017, titled "IMMUNOASSAY METHODS FOR DETECTING THC IN BREATH"; U.S. Provisional Application No. 62/557,060, filed Sep. 11, 2017, titled "DIAGNOSTIC AND ANALYTICAL ASSAY PERFORMANCE FOR THC IMMUNOASSAY"; U.S. Provisional Application No. 62/616,380, filed Jan. 11, 2018, which is titled "METHOD AND DEVICE FOR MEASURING THC LEVEL FROM BREATH SAMPLE"; U.S. Provisional Patent Application No. 62/337,286, filed May 16, 2016, and titled "BREATH COLLECTOR MODULE"; U.S. Provisional Patent Application No. 62/351,858, filed Jun. 17, 2016, and titled "COMPOSITIONS AND METHODS FOR DETECTION OF TARGET CONSTITUENT IN EXHALED BREATH"; U.S. Provisional Patent Application No. 62/351,821, filed Jun. 17, 2016, and titled "SYSTEM AND METHOD FOR TARGET SUBSTANCE IDENTIFICATION"; U.S. patent application Ser. No. 15/217,151, filed Jul. 22, 2016, and titled "COMPOSITIONS AND METHODS FOR DETECTION OF TARGET CONSTITUENT IN EXHALED BREATH"; U.S. Provisional Patent Application No. 62/351,858, filed Jun. 17, 2016, and U.S. patent application Ser. No. 14/997,405, titled "METHOD, DEVICE AND SYSTEM FOR TARGET SUBSTANCE DETECTION" and filed Jan. 15, 2016, U.S. Provisional Application Nos. 62/104,813, filed Jan. 18, 2015, and 62/107,331, filed Jan. 23, 2015, both of which are titled "METHOD, DEVICE AND SYSTEM FOR TARGET SUBSTANCE DETECTION," U.S. Provisional Application No. 62/277,854, filed Jan. 12, 2016, and titled "PORTABLE, HAND-HELD INSTRUMENT FOR DETECTION AND QUANTIFICATION OF CANNABINOIDS AND ALCOHOL IN EXHALED HUMAN BREATH," and U.S. Provisional Application Nos. 62/508,864, filed May 19, 2017, and 62/514,618, filed Jun. 2, 2017, both of which are titled "SYSTEM AND METHOD FOR TARGET SUBSTANCE IDENTIFICATION."

In the description, for purposes of explanation and not limitation, specific details are set forth, such as particular embodiments, procedures, techniques, etc. in order to provide a thorough understanding of the present technology. However, it will be apparent to one skilled in the art that the present technology may be practiced in other embodiments that depart from these specific details.

While specific embodiments of, and examples for, the system are described above for illustrative purposes, various equivalent modifications are possible within the scope of the system, as those skilled in the relevant art will recognize. For example, while processes or steps are presented in a given order, alternative embodiments may perform routines having steps in a different order, and some processes or steps may be deleted, moved, added, subdivided, combined, and/or modified to provide alternative or sub-combinations. Each of these processes or steps may be implemented in a variety of different ways. Also, while processes or steps are at times shown as being performed in series, these processes or steps may instead be performed in parallel, or may be performed at different times.

While various embodiments have been described above, they have been presented by way of example only, and not limitation. The descriptions are not intended to limit the scope of the present technology to the forms set forth herein. To the contrary, the present descriptions are intended to cover such alternatives, modifications, and equivalents as may be included within the spirit and scope of the present technology as appreciated by one of ordinary skill in the art. Thus, the breadth and scope of a preferred embodiment should not be limited by any of the above-described exemplary embodiments.

What is claimed is:

1. A handheld breath sample apparatus for detection of tetrahydrocannabinol (THC) in a breath sample using Surface-Enhanced Raman Spectroscopy (SERS), the apparatus comprising:

a housing comprising a cartridge interface configured to mechanically interface with a cartridge that has a Surface-Enhanced Raman Spectroscopy (SERS)-active substrate, wherein the SERS-active substrate comprises one or more capture sites for a breath sample; and

a detection device optically coupled to the SERS-active substrate to detect Raman signals emitted from the SERS-active substrate, and to correlate the Raman signals to a threshold level of THC; and

wherein the threshold level of THC is correlated with an average amount of THC in breath between 2 and 3 hours after inhalation.

2. The apparatus of claim **1**, wherein the SERS-active substrate comprises metal nanoparticles.

3. The apparatus of claim **2**, wherein the metal nanoparticles are at least one of gold nanoparticles and silver nanoparticles.

4. The apparatus of claim **3**, wherein the THC present in the breath sample is absorbed or adsorbed at or in the capture sites.

5. The apparatus of claim **4**, wherein a surface of the SERS-active substrate is configured to cause excitement of localized surface plasmons upon exposure to a laser light, thereby enhancing the Raman signals and allowing for trace detection of THC.

6. The apparatus of claim **5**, wherein the enhancing of the Raman signals is 10^3 to 10^{10}-fold signal increase, as compared to traditional "bulk" Raman scattering, by a strong electromagnetic wave coupling of the Raman signals.

7. The apparatus of claim **6**, wherein the trace detection of THC is single molecule detection of THC in the breath sample.

8. The apparatus of claim **1**, wherein the SERS-active substrate is configured to facilitate droplet capture through inertial impaction.

9. The apparatus of claim **8**, further comprising:

an impaction port disposed within the housing, wherein the impaction port has a longitudinal axis that is perpendicular to a major plane of the SERS-active substrate, and wherein the impaction port is configured to be in fluidic communication with the one or more capture sites disposed on a surface of the SERS-active substrate.

10. The apparatus of claim **9**, further comprising:

an interface or a laser source configured to optically access the one or more capture sites.

11. A method using Surface-Enhanced Raman Spectroscopy (SERS) for detection of tetrahydrocannabinol (THC) in a breath sample, the method comprising:

determining an amount of THC captured from a breath sample using SERS using a SERS-active substrate, wherein the SERS-active substrate comprises one or more capture sites for a breath sample;

comparing the determined amount of THC captured from the breath sample to a threshold level for THC in breath;

indicating whether the determined amount of THC captured from the breath sample exceeds the threshold level; and

wherein the threshold level of THC is correlated with an average amount of THC in breath between 2 and 3 hours after inhalation.

12. The method of claim **11**, wherein the determining an amount of THC captured from a breath sample using SERS comprises receiving enhanced Raman signals that is 10^3 to 10^{10} fold signal increase, as compared to traditional "bulk" Raman scattering, by a strong electromagnetic wave coupling of the enhanced Raman signals.

13. The method of claim **11**, wherein the SERS-active substrate comprises metal nanoparticles.

14. The method of claim **13**, wherein the metal nanoparticle are at least one of gold nanoparticles and silver nanoparticles.

15. The method of claim **11**, wherein the threshold is correlated with a baseline maximum level of THC in breath associated with consumption of THC outside a window of THC-associated impairment.

16. The method of claim **11**, wherein the determining the amount of THC captured from the breath sample using SERS allows for single THC molecule sensitivity.

17. The method of claim **11**, further comprising:

wirelessly transmitting data corresponding to the determining an amount of THC captured from the breath sample using SERS, the comparing the determined amount of THC from the breath sample to the threshold level for THC in breath, and the indicating whether or not the determined amount of THC captured from the breath sample exceeds the threshold, to a remote location.

18. The method of claim **11**, wherein the THC captured from the breath sample using SERS is captured with a hand-held device.

19. A system using Surface-Enhanced Raman Spectroscopy (SERS) for detection of tetrahydrocannabinol (THC) in a breath sample, the system comprising:

an excitation laser configured to excite a SERS-active substrate, wherein the SERS-active substrate comprises one or more capture sites for the breath sample;

a high sensitivity spectrometer configured to collect one or more Raman signals from the one or more capture sites;

a detector electrically connected to the high sensitivity spectrometer; and

wherein the one or more Raman signals are directly proportional to a threshold level of THC captured in the breath sample, wherein the threshold level of THC is correlated with an average amount of THC in breath between 2 and 3 hours after inhalation.

20. The system of claim **19**, wherein the SERS-active substrate comprises gold nanoparticles, silver nanoparticles, and/or copper nanoparticles.

21. The system of claim **19**, further comprising:

a cartridge, the cartridge comprising the SERS-active substrate including the one or more capture sites for the breath sample.

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